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Department of Soil Science, College of Agriculture, Lorestan University, Khorram Abad, Iran

ABSTRACT

Concentrations of Natural stable and unstable carbon in ecosystems have been used extensively to help to understand a wide range of soil processes and functions. This study was conducted to explore the effects of land use changes on different carbon fractions (F_1, F_2, F_3 and F_4), permanganate oxidizable carbon (POXC), soil organic carbon (SOC) and total organic carbon (TOC) associated with soils in calcareous soils of western Iran. Four popular land uses in the selected site including natural forest, range land, dryland farming and irrigated farming systems were employed as the basis of soil sampling. The results showed a strong relationship between land use conversion and SOC stocks changes. The greatest mean values for carbon content and the least mean values of $CaCO_3$ in bulk topsoil (0–15 cm) in the forest land were observed. Dryland farming had the least both active and passive pools of C in comparison with the other land uses. The positive and significant correlations was observed between SOC, Total C and POXC contents and different C fractions. Taking C and POXC pools into account, a more definitive picture of the soil C is obtained than when only total C is measured. The influence of land use changes on overall soil carbon stocks could be helpful for making management decision for farmers and policy makers in the future, for enhancing the potential of C sequestration in western Iran.

Introduction

Soils are the largest carbon (C) pool in terrestrial ecosystems globally containing more than two-thirds of ecosystem total C. Land-use change and any accompanying soil disturbance can be a major cause of C loss, for example, following deforestation,[1–3] cultivation [4] or conventional farming.[2] Labile soil organic matter (SOM) is considered to have fast decomposition rates and short turnover times. It accounts for only about 5% of total SOM, but is highly active as it consists of readily decomposable compounds.[5] Land use change significantly affects soil organic carbon (SOC) stocks.[2,6,7]

It has been reported that land use change is considered the second most important cause of C emission after fuel consumption.[8] SOC is the most important parameter for the sustainability and quality of ecosystem.[9,10] Natural and anthropogenic factors affect the total SOC storage; and hence the decision for managing agricultural and forest ecosystems influence SOC stocks as a means to mitigate the effects of climate change.[11]

Various methodologies are used for SOC determination. 'Wet chemistry' methods constitute a separate group and are widely used, especially in Russia and East European countries.[12,13] The essence of the method lies in the determination of organic carbon by oxidation with a mixture of potassium dichromate and sulphuric acid. The organic carbon values obtained can be re-calculated into humus or organic matter using the mean coefficient (1.724) or experimentally determined coefficients.[12,14,15] Wide-scale assessment of soil C levels will necessitate the need for very accurate, high-volume analytical procedures capable of measuring incremental changes in soil C. Automated carbon–nitrogen–sulphur (CNS) analysers are commonly used for measurement of soil C; however, inorganic C present as $CaCO_3$ may thermally degrade at high temperatures and be erroneously measured as organic C by CNS analysis.[16] Therefore, pre-treatment of soil with acid is used to remove carbonates, which results in acidic soil residues that may damage instrumentation, interfere with C analysis, and destroy organic matter in samples,[17] in addition to being time consuming. Additional problems associated with CNS analysis include high cost and maintenance. Therefore, the need for alternative approach for accurate and rapid assessment of organic C which limits interference by inorganic C is highly acknowledged.

The loss-on-ignition (LOI) method is an inexpensive alternative to CNS analysis that is reliable and suitable for soil C analysis.[18,19] For the LOI method, soil is oxidised at a high temperature, and the mass loss is proportional to

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CONTACT M. Feizian ✉ feizian.m@lu.ac.ir

Table 1. Selected chemical and physical properties of samples.

Land use	pH	Texture	CaCO$_3$ (%)	% clay	% silt	% sand
Rangeland	7.95	Loam	23.25	19.2	43.2	37.6
Irrigated farming	7.24	Clay loam	32.65	31.2	20.8	48.0
Dry land farming	7.09	Clay loam	32.75	33.2	25.2	41.6
Forest	7.17	Clay loam	9.37	39.2	20.8	40.0

the organic matter content of the soil. Carbonates remain stable at temperatures used for analysis and thus are not measured by the LOI method.[19] The strong correlation between combustion or wet oxidation and loss-on-igni-tion (LOI$_{105-350°C}$) had been reported by several researchers. [20–22] The LOI method measures the organicmatter con-tent of soils, but the conversion of the organicmatter con-tent to a C basis may be difficult because the C content of organic matter is not consistent and varies with vegetation type, degree of decomposition, age, and other factors.[23]

The objectives of this study were to assess the organic C fractions in soils under different land uses and deter-mine the LOI method to the calcareous soils.

Materials and methods

Description of studies

The study area is located in the Razan region of Khorram-Abad district, Lorestan Province in western Iran, between 47°31′and 48°57′E longitudes and 32°56′ and 33°51′N lat-itudes. Climate is semiarid in south west Iran. The mean annual temperature and precipitation at the site are 17.7 °C and 350 mm, respectively. The study area was consisted of four different land uses adjacent to each other. All stands are characterised by similar climatic conditions and predominantly classified as Inceptisols. According to geographic map of Lorestan province, the parent material of this region is limestone. Three major land covers and land uses were established in the study area including forest, rangeland, and cultivated land. The cultivated lands include dry land and irrigated land. Different crops are cultivated in croplands such as wheat and other cereals. Rangeland has been subjected to human activities such as traditional animal husbandry and cutting bushes and shrubs for expanding cropland.

All the chosen forest and grassland stands have south-erly aspects, and are on slopes of <30°. We established study plots of 20 m by 20 m in each of the land uses. Each plot was further divided into four subplots (10 m × 10 m). In each subplot, soil samples were collected from four replicated points to reduce the variability due to chance factor. Soil samples from four replicated points in each subplot were mixed for reducing the number of soil samples. Therefore, we obtained four samples for each landuse. The soil samples were air-dried and sieved using a 0.25 mm sieve. Chemical analyses were carried out at the soil science Research Laboratory of Institute of Agriculture, Lorestan University. Prior to analyses, all visible plant and animal residues were removed from soil

samples and then the remaining soil samples sieved using a 0.25 mm sieve. As shown in Table 1, most of the char-acteristics of the selected soils (Ap horizons) displayed a wide range of variation. pH values of soils were up to 7.95, and were characterised, in general, by a high calcium carbonate equivalent. The soils had a clay-loam texture class. It may be noteworthy that all the soil samples were from the same region and subsequently all the samples have the same parent material. Therefore the difference between results derived only from type of landuse.

Each sample was analysed for permanganate oxidiza-ble carbon (POXC), LOI and organic carbon following two different modified-WB methods. Triplicate samples were used to extract all the C fractions and also evaluate the LOI contents of C using the different procedures.

Oxidisable organic C and its fractions

The soil organic carbon (SOC) was determined using wet oxidation (modified-WB) method as proposed by Nelson and Sommers [14]. Its different fractions were estimated through a method described by Chan et al. [24] using 5, 10 and 20 ml of concentrated (36.0 N) H$_2$SO$_4$ resulting in three acid-aqueous solution ratios of 0.5:1, 1:1 and 2:1. This corresponded to 12.0, 18.0 and 24.0 N of H$_2$SO$_4$, respectively. The amounts of C$_{oc}$ thus determined allowed separation of C$_{tot}$ into the following four different fractions of decreasing oxidizability. According to Chan et al. [24] the above fractions are grouped into active$\left[\sum\left(C_{frac1} + C_{frac2}\right)\right]$ and passive$\left[\sum\left(C_{frac3} + C_{frac4}\right)\right]$ pools. The discussion on C stabilization in soils is mostly based on the transformation of C into such active and passive pools.

Total organic carbon

Because of high pH value (>7) we expected the presence of carbonate into the soils. Therefore, the carbon esti-mated by dry combustion method was not considered as organic carbon. Soil samples were also analysed for inor-ganic C (carbonates) using dilute HCl method. Therefore the LOI method was employed for the determination of total carbon.

The LOI procedure described by Nelson and Sommers [14] was used as the basic method. Five gram of sample was placed in 30 ml crucibles that have been pre-weighed and pre-heated at 105 °C, then cooled. Crucibles with soil samples were then handled as follows:

(1) Heat in a drying oven at the initial (prepara-tion) temperature overnight,

(2) Cool to room temperature in a desiccator over silica gel,
(3) Weigh the crucibles + sample to the nearest mg. re-weigh at 105 °C.
(4) Place into a muffle furnace and heat at the ignition temperature for 16 h.
(5) Remove from the furnace and cool to room temperature in a desiccator.
(6) Weigh the ignited samples to the nearest mg and record weight.

The mass of soil loss expressed on a dry weight basis by the following equation:

$$LOI\left(g\,kg^{-1}\right) = (\text{Weight initial T} - \text{Weight ignition T})/$$
$$\text{Weight initial T} \quad \times 1000.$$

The loss in weight of the soil is taken as a raw measure of the organic carbon content. An initial temperature of 105 °C and ignition temperature of 350 °C were employed for this study. The measurements were duplicated.

Permanganate oxidizable carbon

All POXC analyses were based on Weil et al. [25]. A detailed protocol of this method can be found at: http://lter.kbs.msu.edu/protocols/133 (verified 6 January 2012). Briefly, 2.5 g of air-dried soil were weighed into polypropylene 50-mL screw-top centrifuge tubes. (Note: The method originally published by Weil et al. [25] used 5.0 g of soil, but 2.5 g is now recommended.) To each tube, 18 ml of deionized water and 2 ml of 0.2 MKMnO$_4$ stock solution were added and tubes were shaken for exactly 2 min at 240 oscillations per minute on an oscillating shaker. Tubes were removed from the shaker and allowed to settle for 10 min. (Shaking times and settling times are very important with this method, so batches of 10 samples or less were run.) After 10 min, 0.5 ml of the supernatant were transferred into a second 50-ml centrifuge tube and mixed with 49.5 ml of deionized water. An aliquot (200 μl) of each sample was loaded into a 96-well plate containing a set of internal standards, including a blank of deionized water, four standard stock solutions (0.00005, 0.0001, 0.00015, and 0.0002 mol l^{-1} KMnO$_4$), a soil standard and a solution standard (laboratory reference samples). All internal standards were analytically replicated on each plate. Sample absorbance was read with a *SpectraMax M5* using Soft max Pro soft ware (Molecular Devices, Sunnyvale, CA) at 550 nm. Permanganate oxidizable C was determined following Weil et al. [25] equations. Each sample was analysed in triplicate and the average reported.

Statistical analysis

All data were analysed using the SPSS 22 for Windows software package (2013). The data were tested for homogeneity of variances (Brown & Forsythe's variation of Levene's test) before further testing. No transformation was done since data met the assumption of homogeneity of variances. Post hoc mean separation was evaluated using Tukey's multiple comparisons and one-way ANOVA was used to identify the differences of C fractions, C stocks and POXC in bulk soil and each C fraction among rangeland, forest, irrigated farming and dry farming land. A Spearman correlation (n = 16) was used to test the relationship among C concentration obtained by different methods and relative C content in the four different land uses. For all data, all differences reported in the text were tested and considered significant at α = 0.05.

Results and discussion

Effect of landuse on the oxidizable distributions of carbonand LOI

Figure 1 shows that C concentration in SOC and LOI in the top soil varied among land uses. The highest C concentration (12.17%)was observed in the forest while the lowest (4.90%) concentration was obtained for dryland farming. SOC values varied from 0.8 to 2.46% in modified WB and 2.74 to 6.86% in LOI methods between deferent landuses (Table 2). The corresponding recovery values for SOC ranged from 28.8 to 43.2%. The differences in C concentrations (LOI and SOC) between rangeland, forest, irrigated farming and dryland farming were statistically significant and showed the following order: forest land > irrigated farming > range land > dry farming land (Figure 1).

As expected, C concentrations of LOI were highest in the forest land, intermediate in irrigated farming and range land and lowest in the dryland farming.

Results showed that the relative contents of C in the various fractions (F_1, F_2, F_3, and F_4) in the soils differed markedly across samples, reflecting the combination of acid concentrations and C extraction changes (Table 3).
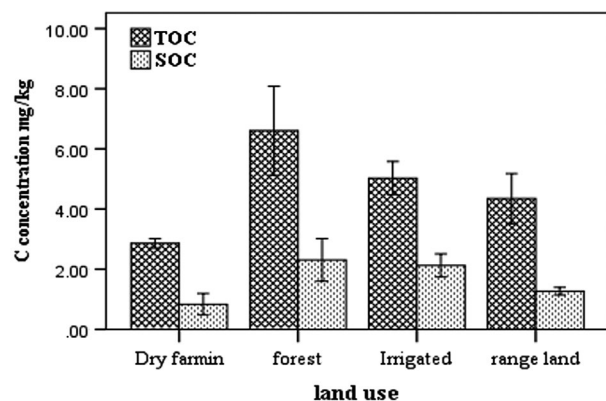

Figure 1. Average content of soil organic carbon and LOI values among different land uses. Bars on the columns stand for standard deviations.

Table 2. Mean total and oxidizable carbon by two different methods (SOC = oxidizable carbon by modified Walkley-Black method and POXC = oxidizable carbon by potassium permanganate).

Variables	Rangeland	Irrigated farming	Dry farming	Forest
TOC	7.81bc	8.8b	4.90c	12.17a
SOC	1.28b	2.23a	0.80b	2.46a
POXC	0.65c	0.86ab	0.33c	1.03a

Note: Different letters within each column indicate the significant difference of mean values ($p < 0.05$) among land uses.

Table 3. Fractionation of organic carbon (%) from soil under different land use using a modified Walkley-Black method Chan et al. (2001).

Land use	F_1	F_2	F_3	F_4
Rangeland	0.70(0.15)[bc]	0.16(0.12)[ab]	0.32(0.17)[a]	3.16(0.55)[b]
Irrigated land	1.20(0.24)[ab]	0.35(0.08)[a]	0.66(0.33)[a]	2.96(0.5)[bc]
Dry farming	0.46(0.18)[c]	0.08(0.03)[b]	0.28(0.05)[a]	1.98(0.25)[c]
Forest	1.50(0.31)[a]	0.22(0.44)[ab]	0.72(0.23)[a]	4.32(0.56)[a]

Notes: Values within parenthesis are SD. Different letters within each column indicate the significant difference of mean values ($p < 0.05$) among land uses ($F_1 = 12$ N H_2SO_4; $F_2 = 18$ N–12 N H_2SO_4; $F_3 = 24$ N–18 N H_2SO_4; $F_4 = TOC$-24 N H_2SO_4).
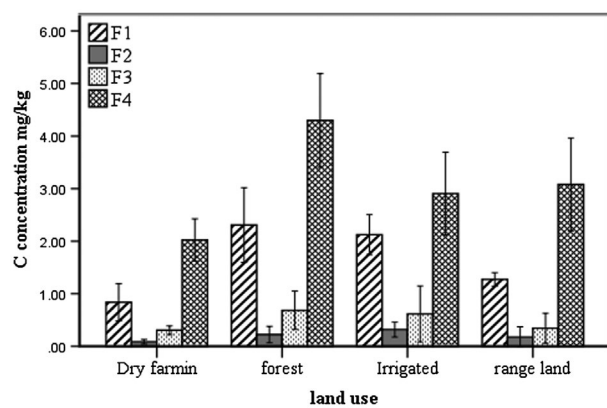
Figure 2. Average soil carbon fractions among four different land uses. Bars on the columns stand for standard deviations.

Applying this method, the results of C fractions did not differ from those of the previous methods (Table 3).

The relative content of C in the F_1 and F_2 differed significantly across dryland and irrigated farming land uses (Figure 2). Significant difference also observed in the F_1 and F_4 of forest land with corresponding fractions in the other land uses (Table 3). From Table 3, the passive and active pools of C in four different land uses can be calculated. The passive pool which represents the stable form of carbon, showed higher amount of C that included 3.5, 3.62, 2.26 and 5.1% for range, irrigated, dry farming and forest lands respectively. The forest land showed that the highest amount of passive pool of carbon, while the lowest obtainedfor dryland farming.

It could be noticed that when modified WB, POXC or fractionation protocols were followed, SOC was under-estimated and the SOC recovery percentage was varying among different samples. The greatest mean values for carbon content and least mean values of CaCO$_3$ were for forest land due to high organic residues and tree litter. Dryland farming had the smallest average values for OM (Figure 1). Sparse vegetation and low amounts

of organic matter returned to the soil might be reasons for the low OM content in dryland farming. There was no significant difference in carbonate content among the land uses, probably because this property is dependent primarily on geological properties and not on management practices (Figure 2).

Wright et al. [26] reported that at less than 400 g kg^{-1}, total C analysis was impaired by the presence of CaCO$_3$, and the best predictive models relating LOI to total C included total Ca as a component. Although total C by CNS analysis and LOI both reflect the soil C status at high organic matter levels, LOI was the best measure of the C status in wetlands with lower organicmatter contents (<400 g kg^{-1}), because of lack of interference of CaCO$_3$.

Ben-Dor and Banin [20] reported that the loss of mass from the soil might be subdivided into several stages:

(1) Hygroscopic water loss (50–100 °C),
(2) Organic matter loss (100–400 °C),
(3) Thermal reactions such as dehydroxylation of phyllosilicates (200–700 °C),
(4) Decarboxylation of carbonates (700–1000°C).

Konare et al. [21] reported that the Adjusted R^2 of the comparison of LOI at 600 °C with the combustion estimate of C was much lower than the one at 350 °C.

The higher uncertainty of LOI estimates at the ignition temperature 600 °C was probably due to dehydroxylation and decomposition of inorganic constituents resulting in increased weight losses and subsequent inaccurate estimate of organic matter content.[27,28] The uncertainty is more pronounced with high clay soils that contain gibbsite with small amounts of soil carbon, particularly in subsoils.[27,28] These authors point out that gibbsite tends to lose much water at 300 °C. Thus, the discrepancy could be due to the fact that hydrated aluminosilicates, carbonate minerals, and some hydrated salts are decomposed at the high temperatures.[20,28]

Our results corroborated those obtained by previous researchers, who also found that Oak–evergreen cloud forest had the largest amounts of SOC at all depths.[29]

Karchegani et al. [30] showed that natural Oak forest had higher SOC content compared to the disturbed forest and cultivated soils in steep lands of Lordegan district, western Iran. These findings indicate that the forest land had the highest stock of C in comparison with the other land uses. Havaee et al. [31] reported that the greatest OM were measured in irrigated farming and dryland farming had the smallest OM content in the calcareous soils of Semirom region, central Iran. Many have noted the influence of landuse type/change on soil TOC and extractable organic C content.[32–37]

Although most of these studies have examined soil C pools before and after landuse change, a few have contrasted the soil C pool characteristics of a number of current and stable landuses. For example, forest soils showed 6 and 8% more TOC than crop [37] and tree plantation soils,[38] respectively. Improved pasture and urban soils were found to contain 8 and 12% more TOC than tree plantation soils.[39] In contrast, this study found greater TOC in forest soils than cultivated and range lands.

Effect of landuse on POXC

POXC values varied from 0.33 to 1.03 gr kg^{-1} between different landuses (Table 2).

In contrast to observation for fractionation method, C concentration which reacted with $KMnO_4$ solution (POXC) varied significantly among land uses in the similar order with the other methods.

Furthermore, to be meaningful as an estimate of the active C pool, the results of the proposed method should exhibit significant relationships with some soilquality indicators. In the current study we presented data to show that the proposed method is sensitive to management-induced soil changes. Weil et al. [25] proved that a $KMnO_4$ solution by the concentration of 0.02 M was adopted as most suitable for the proposed active C method. The sensitivity of the POXC parameter to the perceived differences in soil quality can be judged by the F-value ($F = 17.2^{***}$). A repeatable, easy-to-use method for

estimating active soil C will be helpful in assessing soil quality only to the extent that the C fraction measured is sensitive to changes in soil quality and allows the investigator to detect these changes consistently.

Relationships between SOC, total organic C, POXC and soil properties

Table 4 shows the correlations among soil organic C fractions and some soil properties in surface soil samples collected from four different land uses representing a wide range of cropping systems. The correlation of SOC, Total C and POXC contents with C fractions were positive from moderate to strong and significant at 95–99% probability level (Table 4); conversely, a negative correlation was found between $CaCO_3$ content and C content of soils. It can be seen in the Table 4 that there is no significant correlation between soil clay and organic C concentrations. Several previous studies have revealed strong correlations between soil C content and clay content,[40] but the correlation varied among soils with different iron and aluminium oxide content.

Across the C fractions, F_1 showed the strong correlation with both SOC and POXC. As it can be seen in Table 4, F_4 also correlated strongly with total organic C in comparison with the other C fractions.

Conclusions

The land use changes in particular affect vegetation cover and soil carbon stocks by altering the balance between carbon loss and accumulation. Our findings provided estimates of the effects of land use changes on SOC stocks in a representative area in the semi-arid region in western Iran. The results showed a strong relationship between land use conversion and soil carbon stock changes. In the all four sites, the significant reduction in C content due to dryland farming was observed. By contrast, the irrigated farming system has a higher C content than adjacent dryland farming. At each of the sites examined, dry farming has resulted in a marked decline in both active and passive C pools. The overall C concentration in the top soil was the highest in the forest land. By taking carbon pools and POXC into account,

Table 4. Spearman's correlation coefficients among relative total C, POXC, relative carbon fractions and some intrinsic soil properties (n = 16, four land uses and four soil C fractions).

	Clay	CaCO$_3$	POXC	SOC	TOC	F_1	F_2	F_3	F_4
Clay	1								
CaCO$_3$	−0.35	1							
POXC	0.31	−0.32	1						
SOC	0.29	−0.36	0.93**	1					
TOC	0.35	−0.6	0.91**	0.88**	1				
F_1	0.29	−0.36	0.93**	0.99**	0.89**	1			
F_2	−0.06	−0.11	0.57*	0.58*	0.39	0.58*	1		
F_3	0.30	−0.21	0.51*	0.81**	0.59*	0.73**	0.30	1	
F_4	0.26	−0.76**	0.72**	0.66**	0.94**	0.67**	0.37	0.22	1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

a more definitive picture of the soil C is obtained than when only total C is measured.

Therefore, sound land management is needed to achieve sustainable land uses based on the land suitability to enhance OM and other soil quality attributes. Development of relationships between different C analytical methods and total Ca may help to ascertain the best method for assessing soil C in calcareous soils of Iran. A standard, easily reproducible, accurate, and inexpensive method for soil C analysis is needed to gauge the success of restoration projects and for assessing soil C storage and transformations. Refinement of organic carbon determination methods is aimed to make all analytical procedures user-friendly, safe, time-efficient and economical.

ORCID

M. Feizian [iD] http://orcid.org/0000-0002-3206-4434

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