



ORIGINAL RESEARCH

Assessment of changes in different fractions of the organic carbon in a soil amended by nanozeolite and some plant residues: incubation study

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Abstract

Introduction Organic carbon (OC) fractions play important roles in soil and many ecosystem processes. This study focuses on changing of OC in density and soluble fractions in a soil amended by nanozeolite and plant residues that incubated in lab condition for 90 days.

Results The results showed that amounts of OC in light fraction (LF) and heavy fraction (HF) increased with the increasing percentage of nanozeolite and plant residues in the soil. The highest amounts of LF (7.54 g LF. kg⁻¹_{Soil}) and HF (11.10 g kg⁻¹_{Soil}) were found when 30 % nanozeolite and 5 % wheat and alfalfa straws were added to the soil. Accordingly, wheat and alfalfa straws were effective on increasing the LF and HF, respectively. However, they decreased with declining the OC from the 1st day of experiment until the 90th day of experiment. Soluble OC in hot (2.22 g kg⁻¹_{Soil}) and cool (1.54 g kg⁻¹_{Soil}) water fractions increased by addition of 30 % nanozeolite and 5 % plant residues particularly alfalfa straw in comparison with control. Although these soluble fractions increased after initial 30 days of incubation, they decreased in the continuation of the experiment.

Conclusion In fact, OC contents in density and soluble fractions increased by addition of 30 % nanozeolite and 5 % plant residues into the soil; however, they decreased in

initial 30 days of incubation with declining the OC. The findings of this research revealed the application of nanozeolite and plant residues improved carbon pools in density and soluble fractions and carbon sequestration increased by increasing OC contents in soil.

Keywords Alfalfa straw · Wheat straw · Light fraction · Heavy fraction · Hot water · Cool water

Introduction

It has been recognized in last decades that the amounts of carbon stored in soils have important effects on a global scale. Therefore, land management practices affecting soil organic carbon (SOC) content may have a global impact, if they are applied over large areas (Bronick and Lal 2005). Therefore, the small fluctuations of SOC may potentially alter the atmospheric carbon dioxide (CO₂) concentration and the global climate (Mahmoodabadi and Heydarpour 2014). The carbon storage and rate of CO₂ sequestration in soils depend on climate, soil properties and management. Soils as a sink for atmospheric CO₂ play a key role in the global carbon budget as well as in the global carbon cycle (Eshel et al. 2007). Soils are known as one of the largest active carbon pools after the hydrosphere and the lithosphere. The role of soils as either a source or a sink for greenhouse gases, in general, and that of CO₂, in particular, has been a focus of recent studies (Majumder et al. 2008; Bhattacharyya et al. 2009).

Whereas the largest terrestrial pool of carbon is located in the soils (Bhattacharyya et al. 2009), there are many factors that influence on carbon retention and release in soil and also carbon exchange between soil and atmosphere (Majumder et al. 2008). Storage of organic carbon (OC) in

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agricultural systems is a balance between carbon additions from non-harvested portions of crops (Wang et al. 2011), organic sources (Thelen et al. 2010) and carbon losses, primarily through organic matter decomposition and release of respired CO₂ to the atmosphere (Bird et al. 2002). Last researches have shown that organic substances improve soil aggregation and consequently soil structure and also diminish soil compaction and surface crusting, enlarge carbon sequestration and nutrient availability, and increase infiltration and water-holding capacity (Balashov et al. 2010; Aminiyani et al. 2015).

Density fractionation

Physical fractionation, in contrast to chemical fractionation, allows for isolation of fractions, can isolate as intact as possible the SOC associated predominantly with soil minerals (primary organo-mineral complexes) and also the SOC protected within aggregates (secondary organo-mineral complexes) due to their three-dimensional architecture (Straathof et al. 2014). OC fractions exhibit different rates of biochemical and microbial degradation (Stevenson 1994; Tan et al. 2007) as well as different accessibility and interactions (Sollins et al. 1999). Very little is known of the dynamics of soil organic matter (SOM) after agricultural abandonment. SOC contains fractions with a rapid turnover rate as well as fractions with a slower turnover rate (Aminiyani et al. 2015). The labile fractions of OC, such as microbial biomass carbon (MBC) and dissolved organic carbon (DOC), can respond rapidly to changes in C supply. The dynamics of SOC are usually described by dividing SOM into two or more fractions. Density fractionation, that is a laboratory procedure, physically separates soil into light (LF) and heavy fractions (HF) (Wander and Traina 1996; Sollins et al. 1999). The procedure is useful method for studying labile pools of SOC that are more sensitive to cropping practice than is the total SOC pool in temperate soils (Janzen et al. 1992). Generally, sodium polytungstate (SPT) (1.85 g mL⁻¹) and NaI (1.3 g cm⁻³) solutions are often used for density fractionation (Golchin et al. 1994; Magid et al. 1996; Six et al. 2002). Although LF is commonly referred to a plant-like and less stable fraction with high OC concentration (Gregorich et al. 2003), HF is more stable and high-density organo-mineral fractions having lower C concentrations (Golchin et al. 1995a, b).

Light fraction of SOM not only is sensitive to changes in management practices (Cambardella and Elliott 1992; Bremer et al. 1994) but also correlates well with the rate of nitrogen (N) mineralization (Hassink 1995; Barrios et al. 1996). The importance of LF (including free and occluded organic C within aggregates) is widely recognized for its role in formation and stability of soil structure, especially in stabilization of soil macroaggregates (>250 μm) (Miller

and Jastrow 1990; Kay 1998). Janzen et al. (1992) concluded that LF in surface soil (0–7.5 cm) accounted for 2–17 % of SOC, depending principally on cropping systems. However, there are few studies about these two fractions and their contributions to OC storage as related to changes soil management systems.

Water-soluble organic carbon

Dissolved organic carbon (DOC) is defined as a mixture of organic molecules produced by the decomposition of SOM and plant material and by root exudation (Strobel et al. 2001). Although it has been assumed that dissolved organic matter (DOM) represents a labile part of SOM and that total DOC concentration and especially its easily degradable part resembles soil microbial activity, it has been suggested that a great part of DOM in soil represents a relatively stable by-product of microbial activity (Zhao et al. 2008; Wang et al. 2013). The movement of DOM is significant to the cycling and distribution of nutrients and carbon within and between ecosystems and contributes to soil forming processes (Kalbitz et al. 2003). Plant residue and humus are the most significant sources of soluble organic matter in soil. Gregorich et al. (2000) hypothesized that, although the water-soluble carbon pool was small, it had a high turnover and was in equilibrium with soil humus. Liang et al. (2012) suggested that the difference in water-soluble and biodegradable C in agricultural soils was greater than forest soils due to increases in soluble humic materials in agricultural soils. Therefore, humus is probably the main source of DOC because of the relatively large amount of humus present in soil relative to that contributed by the microbial biomass or recently deposited plant residues. DOC inputs to soil solution originate from biological decomposition, throughfall or litter leaching, root exudates and from deposition of soot and dust (Wang et al. 2013).

Laboratory studies (Smolander and Kitunen 2011; Liang et al. 2012; Kiikkilä et al. 2014) have shown that microorganisms can decompose many amounts of water-soluble organic matter fraction. These studies, which ranged in duration from hours to months, indicated that 10–40 % of the water-soluble OC was decomposable under laboratory conditions. Liang et al. (2012) reported that SOC had significantly positive correlations with labile organic C fractions in the 0–20 cm depth. Smolander and Kitunen (2011) observed positive correlations between DOC concentration extracted from soil and the rate of C and net N mineralization and amount of C and N in microbial biomass, which were used to assess soil microbial activity. High temperature is known to hydrolyze organic structures, lyse cells and dissociate



organic materials from inorganic colloids (Kiikkilä et al. 2014). Kim et al. (2014) reported strong correlations between organic C extracted with hot water and mineralizable carbon. This easily degradable DOM seemed to decrease fairly consistently during the degradation of organic matter as observed previously (Don and Kalbitz 2005; Sanderman et al. 2008).

During last years, great strides have been made in a number of research topics including characterizing the spatial and temporal variations in the concentration and flux of DOC reviewed by Kalbitz et al. 2000 and Aitkenhead-Peterson et al. 2003, quantifying its role in soil chemistry and pedogenesis (e.g., Kaiser and Zech 1998; Jansen et al. 2003; Cances et al. 2003), describing the chemical composition of DOC (Guggenberger and Zech 1994; Kaiser and Zech 1998; Strobel et al. 2001), and quantifying the availability of DOC to soil microflora (Zsolnay and Gorlitz 1994; Yano et al. 2000; Kalbitz et al. 2003; Marschner and Kalbitz 2003). There are few studies about assaying of zeolitic materials effects on SOC. Zeolitic materials are extensively used to improve soil physical environment, particularly, in sandy and clay poor soils (Abdi et al. 2006). The assessment of nanozeolite effects on SOC showed that the addition of higher percentage of nanozeolite with alfalfa straw into the soil increased SOC pools and improved soil aggregation stability (Aminiyan et al. 2015). The main objectives of this study were to determine water-soluble and density fractions of OC in soils that treated by different percentage of nanozeolite and some plant residues and incubation them in field capacity for 90 days.

Materials and methods

Study area

This study was conducted on agricultural soil in Azandariyan, Hamedan province, the west of Iran. This area was located between longitudes 47°42' and 48°45' E and latitudes 33°28' and 34°29' N. The climate of the region is semiarid with a mean annual precipitation of 300 mm and a mean annual temperature of 10 °C. The soil of the area is mostly classified as Typic Haplocalcids (Aminiyan et al. 2015).

Sampling, treatment and analysis of soil

The methods used for soil sampling, treatment and analysis were reported in Aminiyan et al. (2015). The treated and moistened soils were incubated in laboratory condition (20–25 °C) for 90 days. After 1, 5, 10, 20, 30, 45, 60, 75 and 90 days of incubation, a portion of each soil was taken for the study of in density (light and heavy) and soluble (hot water and cool water) OC fractions.

Density fractionation

About 10 g dried sample was transferred to a 20-ml graduated centrifuge tube, and 50 ml of NaI solution ($d = 1.3 \text{ g cm}^{-3}$) was added. Suspensions were immediately centrifuged at 3000 rpm for 10 min. The supernatant containing the LF was decanted onto Whatman no. 50 filters (2.7- μm retention) and vacuum-filtered. The HF residue was re-suspended twice in fresh NaI solution, and the LFs were combined. LF and HF were then washed four times into pre weighed tins with deionized water, afterward dried at 55 °C for 24 h in the oven, and weighed (Sollins et al. 1999). Then OC content in HF was determined by Walkley and Black (1934) method.

Soluble water organic carbon fractions

The soluble water OC in the whole soil and the three aggregate fractions were extracted using cold water followed by hot water. Soluble organic matter in cold water was extracted from soils by adding 150 ml of distilled/deionized water to a tube containing 15 g of air-dried whole soil or aggregate fraction. The soil water suspension was shaken for 30 min and centrifuged at 4500 rpm for 20 min. The supernatant solutions were decanted and passed through a 0.45- μm cellulose nitrate filter. The weight of extraction tubes with remaining wet soil was recorded in order to calculate the amount of cold water extract remaining. Hot water-soluble organic matter was extracted from these soils by adding water to the wet soil remaining in each tube to return the water volume to 150 ml, then by placing the tubes in a water bath at 80 °C for 16 h. After this period of time, the samples were centrifuged, decanted and filtered as above. Filtered solutions were stored in a refrigerator (4 °C) prior to incubation (Gregorich et al. 2003). Then OC content in HF was determined by Walkley and Black (1934) method.

Table 1 Some of chemical and physical properties of applied soil

EC (dS m ⁻¹)	pH	CEC (Cmol ⁺ kg ⁻¹ soil)	Total organic C (g kg ⁻¹)	CCE ^a (%)	Sand (%)	Clay (%)	Silt (%)
1.1	7.2	4.80	3.41	1.79	69	12	19

^a Carbonate calcium equivalent

Table 2 Some properties of applied plant residues in this study

	pH	EC (dS m ⁻¹)	Total organic carbon (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)	Total phosphorous (g kg ⁻¹)	C/N	C/P
Alfalfa straw	6	9.5	511	22	5.98	23.30	85.20
Wheat straw	7.97	4.3	532	7	4.31	90.75	123.50

Table 3 Some properties of applied nanozeolite

EC (dS m ⁻¹)	pH	Organic C (g kg ⁻¹)	CEC (Cmol ⁺ kg ⁻¹)
0.98	7.17	1.03	400.39

Table 4 Nanozeolite compositions and their %wt

Composition	%wt
SiO ₂	69.44
Al ₂ O ₃	11.87
Fe ₂ O ₃	1.30
Ti ₂ O	0.18
K ₂ O	1.31
Na ₂ O	0.68
CaO	3.28
MgO	0.99

Statistical data analysis

The experiment was a completely randomized factorial design with three replicates. The factors applied were alfalfa straw (0 and 5 %), wheat straw (0 and 5 %), nanozeolite (0, 10 and 30 %) and incubation time (1, 5, 10, 20, 30, 45, 60, 75 and 90 days). All statistical analyses

Table 5 Analysis of variance (mean square) of the effects of nanozeolite, plant residues application, incubation time and their interaction on LF and HF, soluble OC in cool water and hot water fractions in soil

Source	DF	Organic carbon fractions			
		LF (g LF. kg ⁻¹ Soil)	OC in HF (g kg ⁻¹ Soil)	Soluble OC in cool water (g kg ⁻¹ Soil)	Soluble OC in hot water (g kg ⁻¹ Soil)
Nanozeolite	2	643.18**	1495.55**	6.70**	28.16**
Plant residues	3	49.63**	165.98**	0.14**	1.48**
Time	8	134.34**	354.49**	3.20**	9.42**
Nanozeolite × time	16	42.76 ns	2.24 ns	1.53 ns	5.08 ns
Plant residues × time	24	34.96 ns	0.95 ns	0.74 ns	2.28 ns
Nanozeolite × plant residues	6	130.61**	2.63**	8.26**	5.63**
Nanozeolite × plant residues × time	48	23.94 ns	0.59 ns	0.37 ns	1.28 ns
Error	216	8.02	0.6	0.3	0.1

** Mean square of the treatment is significant at the 0.01 level

ns Mean square of the treatment is not significant

were performed in the SAS ver.9.2 statistical framework; to obtain the main differences between the treatments, the Duncan's ($\alpha = 0.01$) test was applied.

Results and discussion

Table 1 shows some of chemical and physical properties of applied soil. According to the sand, clay and silt contents, the soil texture was loamy sand. Table 2 presents some properties of applied plant residues. Alfalfa and wheat straw had neutral pH, high OC values and C/N and C/P ratios. Some of applied nanozeolite properties are given in Table 3. Also nanozeolite compositions and their weight percentage are shown in Table 4; according to this table, SiO₂ and Al₂O₃ (69.44 and 11.87 %), respectively, had much higher portion than the other compositions.

The effect of nanozeolite and plant residues on OC in density fractions

Table 5 shows the analysis of variance of the effects of nanozeolite, plant residues application, incubation time and their interaction on LF, HF, soluble OC in cool water and hot water fractions in soil ($p < 0.01$). However, the



Table 6 Light fraction and HF content in all of treatment

Treatment	LF (g LF. kg ⁻¹ Soil)	OC in HF (g kg ⁻¹ Soil)
Control	16.32 ± 1.30* g	17.60 ± 3.00 e
N0A5	17.08 ± 1.27 fg	20.50 ± 3.01 d
N0W5	18.04 ± 1.28 ef	19.70 ± 3.02 de
N10PR0	16.50 ± 2.15 ef	17.98 ± 3.02 c
N10A5	19.20 ± 2.13 cd	25.70 ± 3.01 ab
N10W5	19.52 ± 2.14 de	24.60 ± 3.02 bc
N30PR0	16.63 ± 2.10 bc	18.10 ± 3.00 bc
N30A5	21.78 ± 2.12 ab	28.70 ± 3.02 a
N30W5	23.86 ± 2.11 a	27.00 ± 3.01 b

N0A5 (0 % nanozeolite + 5 % alfalfa straw), N0W5 (0 % nanozeolite + 5 % wheat straw), N10PR0 (10 % nanozeolite + 0 % plant residue), N10A5 (10 % nanozeolite + 5 % alfalfa straw), N10W5 (10 % nanozeolite + 5 % wheat straw), N30PR0 (30 % nanozeolite + 0 % plant residue), N30A5 (30 % nanozeolite + 5 % alfalfa straw), N30W5 (30 % nanozeolite + 5 % wheat straw)

The same letters are not significantly different at $p < 0.01$ using Duncan's LSD test

* Mean ± standard deviation

interactions between nanozeolite and incubation time, plant residues and incubation time and nanozeolite, plant residues and incubation time did not have significant effects on mentioned OC fractions in the soil.

Table 6 reveals the OC content in LF and HF increased by the addition of nanozeolite and plant residues ($p < 0.01$). LF value in 30 % nanozeolite plus 5 % wheat straw (N30W5) treatment was greater than the other treatments; as its value 7.54 (g LF. kg⁻¹Soil) was greater than control, because C/N ratio in wheat straw was higher than alfalfa straw, and thus subsequently wheat straw had

lower stage of biodegradation by microorganisms in soil. This fraction of OC decreased from the 1st day (20.61 g LF. kg⁻¹Soil) to the 90th day (16.99 g LF. kg⁻¹Soil) during soil incubation (Fig. 1). The HF value in N30A5 treatment was significantly increased (11.1 g kg⁻¹Soil) in comparison with the control treatment (Table 6). Also this table investigates that N30A5 treatment increased HF (3 g kg⁻¹Soil) and (8.2 g kg⁻¹Soil) in comparison with the N10A5 and N0A5 treatments, respectively.

It is known that the alfalfa straw was more efficiency due to increasing OC in HF than wheat straw in all of the treatments with the similarity percentage of nanozeolite (Table 6). SOC in the LF plays an important role in retaining of cellulase molecule from washing out and nutrition of soil microorganisms and subsequently humus production. Thus SOM quality is an important factor in its disintegration rate (Schmidt et al. 2002; Beheshti et al. 2012). According to Fig. 2, OC in HF had a distinct downward trend from the 1st day (27 g kg⁻¹Soil) until the 90th day (18.4 g kg⁻¹Soil). The recent research on OC decay dynamics showed that LF and HF were decreased during soil incubation (Hassink et al. 1995; Creamer et al. 2012; Aminiyan et al. 2016). The results of Rovira and Vallejo' studies (2003) were in line with those of the present study.

The effect of nanozeolite and plant residues on water-soluble organic carbon fractions

As shown in Table 7, soluble OC contents in hot water and cool water increased by the addition of nanozeolite and plant residues especially alfalfa straw. The results showed that soluble OC in hot water in N30A5 treatment was greater 2.22, 1.36 and 2.06 (g kg⁻¹Soil) than control, N10A5 and N0A5 treatments, respectively (Table 7). Working on

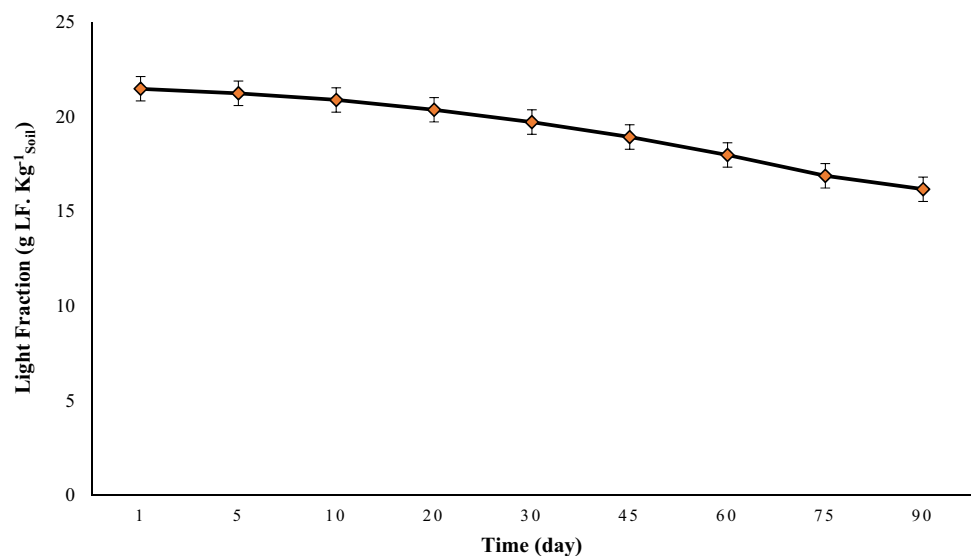
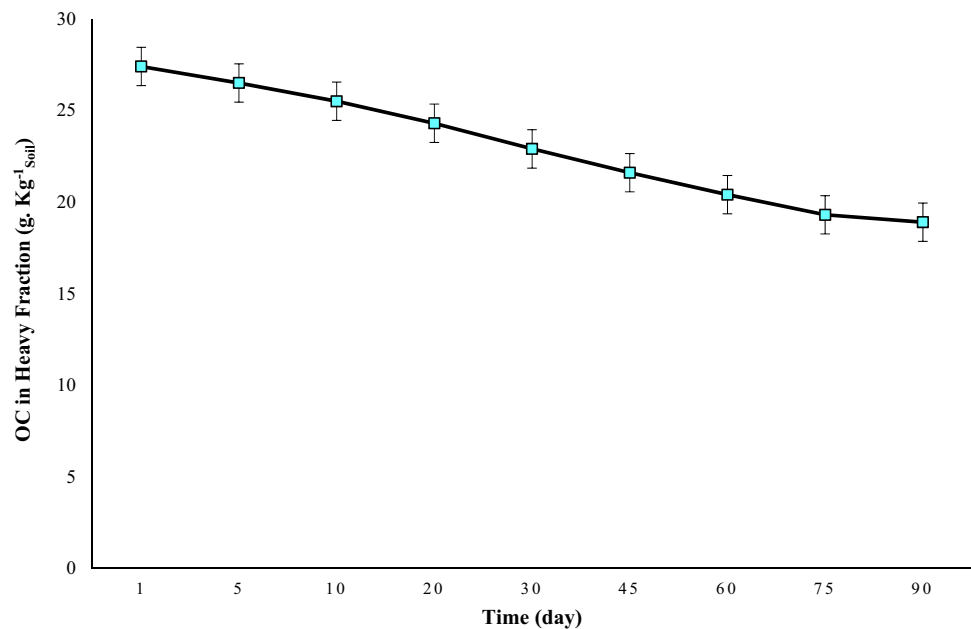
Fig. 1 Light fraction changes with the passage of time

Fig. 2 Organic carbon changes in HF with the passage of time**Table 7** Soluble OC in hot and cool water content in all of treatments

Treatment	Soluble OC in cool water (g kg ⁻¹ Soil)	Soluble OC in hot water (g kg ⁻¹ Soil)
Control	1.93 ± 0.358* c	2.34 ± 0.552 e
N0A5	2.01 ± 0.360 c	2.50 ± 0.546 de
N0W5	1.94 ± 0.357 c	2.42 ± 0.561 de
N10PR0	1.98 ± 0.255 b	2.52 ± 0.454 cd
N10A5	2.33 ± 0.251 ab	3.20 ± 0.461 ab
N10W5	2.29 ± 0.248 ab	3.10 ± 0.477 bc
N30PR0	2.00 ± 0.255 ab	2.63 ± 0.480 ab
N30A5	3.47 ± 0.251 a	4.56 ± 0.491 ab
N30W5	2.51 ± 0.247 ab	3.44 ± 0.473 a

N0A5 (0 % nanozeolite + 5 % alfalfa straw), N0W5 (0 % nanozeolite + 5 % wheat straw), N10PR0 (10 % nanozeolite + 0 % plant residue), N10A5 (10 % nanozeolite + 5 % alfalfa straw), N10W5 (10 % nanozeolite + 5 % wheat straw), N30PR0 (30 % nanozeolite + 0 % plant residue), N30A5 (30 % nanozeolite + 5 % alfalfa straw), N30W5 (30 % nanozeolite + 5 % wheat straw)

The same letters are not significantly different at $p < 0.01$ using Duncan's LSD test

* Mean ± standard deviation

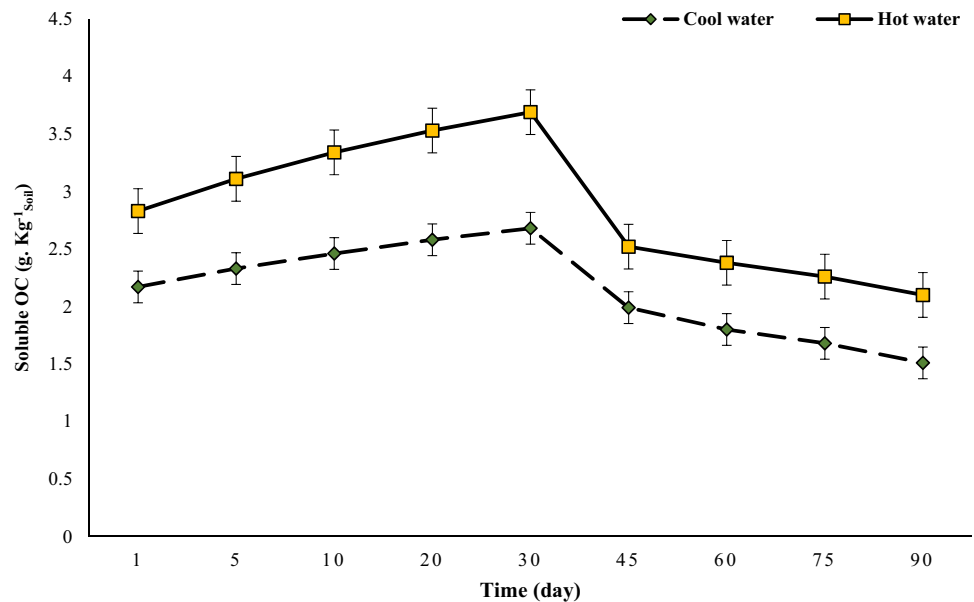
the chemical composition of DOC suggested that most DOC is an end product of microbial metabolism (Guggenberger and Zech 1994); However, short-term experimental manipulations of organic matter sources showed that fresh litter also contributes significantly to the production of DOC (Park et al. 2002). These two views are not necessarily mutually exclusive, but they do point out the considerable difficulty in determining the influence of substrate (litter, SOM), microbial community composition (Muller et al. 1999) and abiotic factors such as temperature and water flux on DOC production and flux (Brooks et al. 1999). Aminiyan et al. (2015) reported that the addition of

30 % nanozeolite with 5 % alfalfa straw to the soil redounded increasing OC in different aggregate particle size classes.

Based on Table 7, the same results were achieved similar to the results of hot water to cool water; accordingly, soluble OC in cool water increased with the greater percentage of nanozeolite and plant residues particularly alfalfa straw. Soluble OC in cool water value increased 1.54 and 0.58 (g kg⁻¹ soil) into the control in N30A5 and N30W5 treatments, respectively. Thus N30A5 treatment more effective to increasing soluble OC in cool water than N30W5 treatment. Soluble OC content in hot water was



Fig. 3 Soluble OC changes in hot and cool water with the passage of time



greater than soluble OC content in cool water (Table 7), because hot water had greater ability to extract of lysis microbial cells and extractable soluble organic matter may be adsorbed to clay or complexed with other organic material produced by plants or decomposing organic matter than cool water (Guggenberger and Zech 1994; Muller et al. 1999). According to the Gregorich et al. (2003) findings, high temperature is known to hydrolyze organic structures, lyse cells and dissociate organic materials from inorganic colloids. The plant residues with lower C/N ratio are a readily decomposable substrate for microorganisms, and they have additional soluble OC content than plant residues with higher C/N ratio (Gregorich et al. 2003). Also these researchers found that the quantity of biodegradable soluble organic matter was related to the extraction procedure and the quantity of organic matter present in the soil.

Figure 3 indicates that soluble OC in both hot water and cool water increased with over time from 1st day until the 30th day of incubation period, but then decreased by the end of the experiment. Accordingly, the soluble OC in cool water increased from 2.17 (g kg⁻¹ soil) in the 1st day to 2.68 (g kg⁻¹ soil) in the 30th day and then it decreased by the end of experiment 1.92 (g kg⁻¹ soil). As shown in Fig. 3, Soluble OC in hot water value increased from 2.83 (g kg⁻¹ soil) in the 1st day to 3.69 (g kg⁻¹ soil) in the 30th day, and finally it decreased by the 90th day 2.39 (g kg⁻¹ soil). Since soluble OC was increased with the development and promoting plant residues biodegradation in the initial 30 days and when the growth and development of microbial communities were increased with the passage of time and subsequently soluble OC decreased with the passage of time. Kalbitz et al. (2003) observed that soluble OC increased

with the passage of time, but in another study soluble OC decreased by over the time (Gregorich et al. 2003). Alfalfa straw had greater soluble OC than wheat straw, and thus its degradation rate and OC content decreasing was done by higher rate in this fraction (Swanston et al. 2002; Preston and Schmidt 2006; Aminiyani et al. 2016). It is known in recent reviews that the organic matter quality is particularly important for SOC stabilization (Amelung et al. 2008; Schmidt et al. 2011).

Conclusion

Organic carbon fractions in soils play important roles in many ecosystem processes. OC fractions exhibit different rates of biochemical and microbial degradation. Density fractionation is a laboratory procedure that separates SOC into LF and HF. Also DOC is affected by the extraction procedure used. Extraction procedures involving higher temperatures extract a greater amount of soluble organic matter than extractions carried out at room temperature. The results of this study showed that LF and HF and water-soluble OC was increased by the addition of greater percentage of nanozeolite and plant residues into the soil. The results of this study showed that LF was greater in N30W5 treatment than in the other treatments. But OC in HF and soluble OC in hot and cool water had maximum amounts in (N30A5) than in the other treatments. LF and HF decreased with the passage of time from the 1st day until the 90th day. Soluble OC in hot and cool water increased from 1st day until the 30th day, and then they decreased by the end of the experiment. In fact, OC content increased by application and addition of nanozeolite and plant residues

into the soil, but these pools decreased with the passage of time. Finally, it can be said that the application of nanozeolite and plant residues improve carbon sequestration process and increase carbon pools in soil.

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