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Yukti Verma
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Nitesh Kumar Singh
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Shakti Om Pathak
Department of Agricultural Chemistry and Soil Science, UP Autonomous College, Varanasi, Uttar Pradesh, India

Correspondence
Yukti Verma
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Application of carbon isotopic techniques in the study of soil organic matter dynamics

Yukti Verma, Nitesh Kumar Singh and Shakti Om Pathak

Abstract
Soil organic matter (SOM) is the earth's largest terrestrial reservoir of carbon which contributes to a variety of biological, chemical and physical properties of soil and is essential for good soil health. The amount (stock) of organic matter in a given soil can increase or decrease depending on numerous factors. But even when stocks are at equilibrium, SOM is in a continual state of flux; new inputs cycle - via the process of decomposition - into and through organic matter pools of various qualities and replace materials that are either transferred to other pools or mineralized. For the functioning of a soil ecosystem, this "dynamics" of SOM is probably more significant than the sizes of SOM stocks. The different carbon isotopic techniques used to study SOM dynamics are: use of organic matter labeled with ^{14}C or ^{13}C, use of natural variation in ^{13}C in organic matter, use of ^{14}C (radiocarbon dating), bomb ^{14}C technique. ^{14}C pulse labeling can be used to trace ^{14}C allocation to shoot and roots, root and rhizomicrobial respiration in grassland communities under drought stress. Based on $\delta^{13}C$ signature of C pools, it is possible to disentangle the contribution of ^{13}C fractionation and preferential substrate utilization of recent (C_4) versus old carbon (C_3) to microbial turnover. ^{13}C isotopic analysis after application of straw is a convenient approach for quantifying carbon-flux partitioning during methanogenic degradation of straw and SOM. The ^{13}C method is generally used in medium-term observations or experiments (5--50 yr); hence, this method gives an estimate of turnover dominated by relatively recent inputs and C pools that cycle within the time frame of the experiment. In contrast, the oldest and most recalcitrant C pools dominate estimates by radiocarbon dating because of the long-term time frame (200-40,000 yr) that this method measures. In future the focus should be on resolving molecular characteristics of organic matter in organo-mineral associations with the knowledge of isotopic signatures of specific molecules. Also the potential of combining isotopic analyses of soil respiration rates with other methods (nutrient uptake in plants) is to be explored.

Keywords: Organic matter, Carbon, Isotopes, Radio carbon dating, Bomb ^{14}C technique

Introduction
Soil organic matter (1580 GtC) constitutes two and half times of biota (620 GtC) and two times that of atmospheric carbon pools (780 GtC). It is in rapid exchange with atmospheric carbon dioxide through the process of photosynthesis and respiration and is thus important as a potential sink and source of green house gases (Gleixner *et al*., 2001) [4]. It has always been into research focus due to combination of its relative large pool size in the terrestrial carbon cycle (Lal, 2010) [8]. However merely knowing the amount of organic matter in soil provides little insight into its role in ecosystem functioning or atmospheric feedbacks since the amount (stock) of organic matter in a given soil depends on numerous factors including climate, vegetation type, nutrient availability, disturbance, land use and management practices. Therefore, for the functioning of a soil ecosystem, the "dynamics" or "turnover" of SOM is probably more significant than the sizes of SOM stocks (Paul, 1984) [10]. Therefore, an understanding of SOM dynamics is crucial for interpretation of data related to ecosystem functioning, soil fertility and global climate change.
The turnover of an element in a pool is most often quantified as the element's mean residence time (MRT) or its half-life ($T_{1/2}$). The MRT of an element in a pool is defined as
1) The average time the element resides in the pool at steady state (Six *et al*., 2002) [13].
2) The average time required to completely renew the content of the pool at steady state.

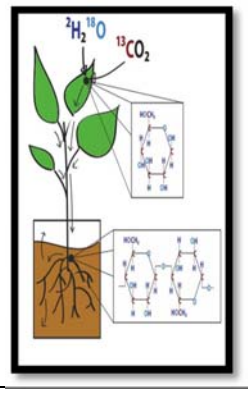
For soils, it is a measure of the first order kinetics for decay ($\tau_o = 1/k$),
At steady state, $\tau_o = M / S$
M is the mass of C in SOM and S is the total C flux from the SOM.

Factors controlling soil organic matter turnover

Primary production (the rate of organic matter transfer below-ground) and soil microbial activity (the rates of SOM transformation and decay) are recognized as the overall biological processes governing inputs and outputs and, hence, SOM turnover. Climate, vegetation type, parent material, topography and time affect production and decomposition. Disturbance or management practices also exert considerable influence on SOM turnover via direct effects on inputs and outputs and through indirect effects on the factors controlling these fluxes.

Table 1: A comparison between conventional techniques and isotopic techniques to study soil organic matter dynamics

Conventional techniques	Isotopic techniques
Disturbs root-soil system	Partitioning of respiration can be done in-situ
Alters soil moisture, microbial species composition and gas diffusivity	Information on soil C dynamics in case of C_3-C_4 vegetation change can be obtained
Little information about recalcitrant pools	Estimate carbon incorporation in pools with low turnover rates.



Carbon isotopic techniques in studying soil organic matter dynamics

Use of organic matter labeled with ^{14}C or ^{13}C

Use of natural variation in ^{13}C in organic matter

Use of ^{14}C (Radio-carbon dating)

Bomb ^{14}C model (Wolf *et al*. 1994) [14].

Use of ^{14}C labeled organic matter

The use of ^{14}C labeled materials to study soil organic matter decomposition has been accomplished by adding radio-labeled plant material, micro-organisms, microbial products or specific compounds to the soil and measuring the amount of $^{14}CO_2$ evolved during anaerobic incubation. ^{14}C labeled plant material can be produced by growing plants in a $^{14}CO_2$ environment in a growth chamber and harvesting them after a suitable growth period. The soil is placed in a flask amended with the ^{14}C labeled organic matter and attached to a CO_2 collection unit. The evolved CO_2 can be trapped in a base such as KOH or NaOH and the ^{14}C activity assayed by liquid scintillation counting techniques. ^{14}C in the sample can be calculated using the formula:

^{14}C in the sample =sample cps-background cps/counting efficiency (Dilution factor) cps- counts per second % Added ^{14}C evolved as $^{14}CO_2$= ^{14}C evolved from sample/^{14}C added to soil (100).

^{13}C natural abundance technique

Approximately 98.89% of all C in the nature is ^{12}C and 1.11% is ^{13}C (Boutton, 1991) [2]. The $^{13}C/^{14}C$ ratio of organic C found in terrestrial environments is determined largely by the isotopic fractionation that occurs during photosynthesis. Plants with C_3 pathway exhibit greater discrimination against ^{13}C than plants with C_4 pathway. These natural differences between plants can be used to study the dynamics of organic matter in soil. Because natural variation in the ratio of $^{13}C/^{12}C$ is small, stable C isotope ratios are expressed in relative terms as $\delta^{13}C_{PDB}$ values:

$$\delta^{13}C_{PDB}\ (‰) = (R_{sample} - R_{PDB}/R_{PDB}) \times 1000$$

where, R_{sample} = mass 45($^{13}C^{16}O^{16}O$) /mass 44($^{12}C^{16}O^{16}O$), R_{PDB}= $^{13}C/^{12}C$ of the international standard (Pee Dee Belemnitella americana limestone).

$\delta^{13}C_{PDB}$ is a relative index that indicates the part per thousand difference between the $^{13}C/^{12}C$ ratio of the sample and that of the PDB standard. Atmospheric CO_2 has a $^{13}C_{PDB}$ value of approximately -8‰. During photosynthesis, plants with C_3 pathway discriminate against atmospheric $^{13}CO_2$ to a greater extent than C_4 plants. C_3 plants have an average $\delta^{13}C_{PDB}$ of -27 ‰, C_4 plants have -13 ‰ and CAM plants have mostly in the range- -20 to -10 ‰. Thus C_3 and C_4 plants have distinct stable isotope ratios and differ from each other by approximately 14‰ on an average. The C isotopic signature of the whole plant is largely preserved as dead plant tissue decomposes and enters SOM pool. The natural isotopic label in the SOM enables reconstruction of the prior history of plant communities and also permits estimation of SOM dynamics in situ over relatively long periods without any type of experimental disturbance.

Radioisotope as a chronometer

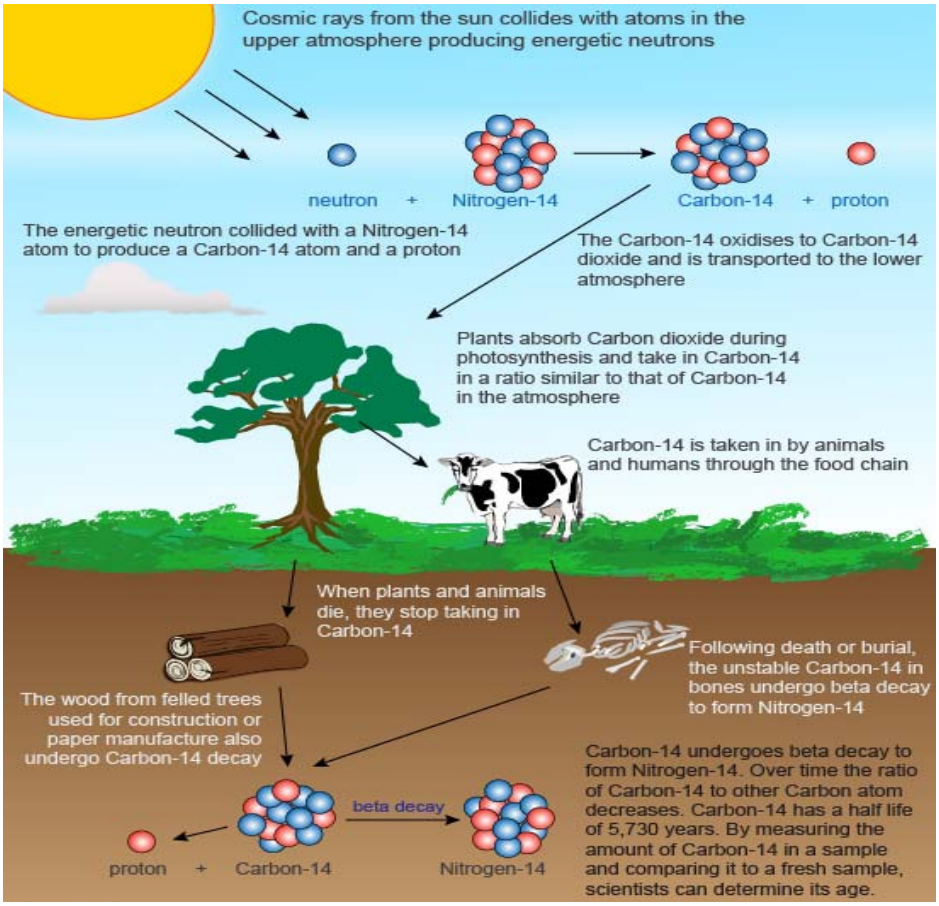


Fig 1: Estimation of age by radiocarbon dating technique Bomb ^{14}C technique.

Thermonuclear bomb tests in the 1950s and 1960s caused the atmospheric ^{14}C content to increase sharply and then to fall drastically after the tests were halted. This sequence of events created an in situ tracer experiment; the incorporation of bomb produced radiocarbon into SOM after the tests stopped allows estimates of turnover of SOM.

Brunn *et al*. (2005) [3] estimated turnovers of soil organic fractions based on different radiocarbon measurements. Three different models were used to estimate turnover of soil organic carbon (SOC) fractions using radiocarbon measurements: one conventional carbon dating model and two bomb ^{14}C models. One of the bomb ^{14}C models uses an atmospheric ^{14}C record for the period 22,050 BC to AD 2003 and is solved by numerical methods, while the other assumes a constant ^{14}C content of the atmosphere and is solved analytically.

Conventional ^{14}C dating model

Theoretical basis for this is the exponential decay of ^{14}C:

$A(t) = A_I(t)\exp(-\lambda a)$

$A(t)$ - Specific activity of the sample corrected for fractionation during photosynthesis by the ^{13}C content

$A_I(t)$ - Specific activity of the sample when it was formed

λ - Decay rate constant of ^{14}C

a - the time since the sample was formed (i.e. its age)

If we assume that the activity of the input is equal to A_{abs} and that all the carbon in the sample entered the soil at the same time, then turn over time (τ):

$\tau = 1/\lambda \ln (A_{abs}/A(t))$

A_{abs}- standard ^{14}C content of new material

Bomb ^{14}C model

The ^{14}C bomb models assume that the turnover of the analyzed SOC fractions is well described by first-order kinetics. Models are applied to SOC fractions. To estimate the model parameters k and I, we use the measured amount of carbon in the SOC fraction, C, and the measured specific activity, A. The amount of C can be found by integrating over the age distribution.

$C = \int \Psi(a)\, da = \int I \exp(-ka)\, da = I/k$ *(numerical solution by solving k)*

Where as the specific activity can be found by

$A(t) = k \int \exp(-(k+\lambda)a)\, A_I(t-a)\, da$ *(analytical solution)*

The turn over time (τ):

$\tau = A_{abs} - A/A\lambda$

From the figure 2 we find that the analytical solution of the bomb ^{14}C is expected to result in better turnover estimates for all values of the specific activity and is just as easy to calculate. As the analytical solution encompasses one simplifying assumption more than the numerical solution (constant ^{14}C content of inputs), numerical solution is expected to be better than analytical solution. The analytical solution produces results that are relatively close to numerical solution for SOC samples of slow turnover. Analytical solution can be used when the TOT of the SOC fraction is sufficiently long and the required precision is not very high.

Conventional ^{14}C dating model effectively assumes that all the C fractions in the SOC fraction is of same age and

therefore, method should be used only in special situation like, layers of peat soils.

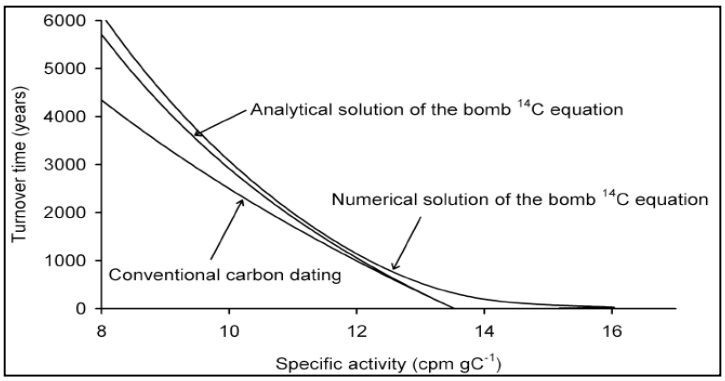


Fig 2: Relationship between three different methods of estimating turnover of SOC fractions.

Application of carbon isotopic techniques in the study of soil organic matter dynamics

Use of organic matter labeled with ^{14} C or ^{13} C

Blagodatskaya *et al*. (2011) [1] estimated the contribution of ^{13}C fractionation and preferential substrate utilization in turnover of SOM and of microbial biomass under C_3 –C_4 vegetation change using ^{13}C natural abundance technique. Results indicated that carbon mean residence time (MRT) based on the proportion of old and recent C in SOM after C_3-C_4 vegetation change was 16.8 years whereas microbial turnover time (MTT) for C_3-C_4 was 29.5 days and was 29.1 days for C_4 indicating that MTT was faster than SOM turnover time.

Gude *et al*. (2012) [6] investigated microbial carbon dynamics of soil organic matter in particle size fractions at different input sites (fresh litter) by analyzing $^{13}C/^{12}C$ isotope ratio of PLFA of microbial community. At the low input site most microbial biomass was found in the (small) particulate organic matter (POM) fraction being dominated by fungal biomass. In contrast, at the high input site the largest amount of microbial biomass was detected in the organo-mineral fraction. Hence availability of fresh carbon led to a greater amount of fungi in POM fraction mineralizing the fresh carbon completely. Fungal activity therefore seems to be essential for incorporation of organic matter input in soil.

Sanaullah *et al*. (2012) [12] studied the effect of drought on C allocation and rhizosphere-mediated CO_2 fluxes under three plant species: Lolium perenne, Festuca arundinacea and Medicago sativa grown in monocultures or mixture using ^{14}C pulse labeling. Greater C allocation to roots than shoots in plant monocultures under drought conditions was found which resulted from lower reductions in root growth compared to shoot growth. There was no significant effect of drought on assimilate accumulation in shoot biomass of *L. perenne.* This plant in contrast increased greatly carbon allocation to its root biomass.

Rodegiero *et al*. (2013) [11] conducted a study on estimation of components of forest soil CO_2 efflux from $\Delta^{14}C$ values of SOM. The study suggested that the average total soil CO_2 efflux was almost equally partitioned between root respiration (50.4%) and SOM respiration (49.6%). The low ^{14}C based decomposition flux from belowground SOM can be reasonably explained by much longer turn over time of light and heavy SOM fractions as compared to litter layers.

Zhang *et al*. (2013) [15] investigated the uptake by microbial community of easily decomposable exogenous organic C and the proportion of this organic C remaining in soils under long-term fertilization schemes using ^{13}C labeled glucose. Following the addition of ^{13}C-glucose, ^{13}C was first incorporated into soil bacterial PLFAs under all three treatment regimes It was attributed to the fact that ample quantities of easily available substrates would quickly be consumed by fast-growing bacteria having higher relative abundance with enzymes that have a high affinity for such substrates. Hence it was attributed that substrate type and relative abundance of microbes affect their response to additive substrate in soil.

Radio Carbon Dating and Bomb ^{14}C technique

Hardie *et al*. (2011) [7] conducted study on abiotic drivers and their interactive effect on radiocarbon distribution within bulk peat and respired CO_2. Results indicated that ^{14}C content of both bulk peat and respired CO_2 decreased with depth. Radiocarbon values >100% Modern unambiguously indicated the presence of bomb-^{14}C (*i.e.* carbon originally fixed after the commencement of atmospheric nuclear weapons tests in the mid 1950s-early 1960s. At each depth, respired CO_2 had elevated levels of ^{14}C relative to the bulk peat (significantly more at 0-10 and 20-30 cm depth) and this finding suggested that selective feeding by decomposer organisms was occurring on younger more labile SOM. These results further demonstrated the vulnerability of peat land carbon stores to environmental change.

Mueller *et al*. (2014) [9] analysed the effect of different stabilization mechanism on ^{14}C signature of different soil fractions. The results indicated that carbon that has been more recently incorporated into a particular fraction is released earlier during incubation whereas carbon incorporated already in the 1960's and 70's (bomb carbon) is released only after 41-180 days.

Range and variation in estimates of total soil organic matter turnover

Although variations within each method are attributable to differences in vegetation, climate, soil type, and other factors, the largest variations in observed MRTs are method dependent. For example, MRTs estimated by ^{13}C natural abundance are generally smaller by an order of magnitude than MRTs estimated by radiocarbon dating, because of the different time scales that the two methods measure. The ^{13}C method is generally used in medium-term observations or experiments (5-50 yr); hence, this method gives an estimate of turnover dominated by relatively recent inputs and C pools that cycle within the time frame of the experiment. In contrast, the oldest and most recalcitrant C pools dominate estimates by radiocarbon dating because of the long-term time

frame (200-40,000 yr) that this method measures (Goh, 1991) [5].

Table 2: Range and variation in estimates of total soil organic matter turnover.

Method and ecosystem	MRT (yr)
^{13}C natural abundance	
• Cultivated systems	61
• Pasture systems	38
• Forest systems	22
Radiocarbon aging	
• Cultivated systems	880
• Forest systems	1005
Bomb ^{14}C analysis	
• Cultivated systems	1863

Turnover of different soil organic matter pools

The MRTs of primary organo-mineral associations generally increase with decreasing particle size, although there are exceptions (particularly among fine gradations of silt- and clay-sized particles) that have been variously related to climate, clay mineralogy, and fractionation methodology. For a given set of biotic and abiotic conditions, the turnover of different SOM pools depends mechanistically on the quality and biochemical recalcitrance of the organic matter and its accessibility to decomposers. With other factors equal, clay soils retain more SOM with longer MRTs than do sandy soils. Readily decomposable materials can become chemically protected from decomposition by association with clay minerals and by sorption to humic colloids. Studies of the average MRTs of organic matter in macro-aggregates vs. Micro-aggregates show consistently slower turnovers in micro-aggregates as shown in table 3. Thus, a much higher proportion of the SOM occluded in micro-aggregates consists of stabilized materials with relatively long MRTs.

Table 3: Turnover of different soil organic matter fractions.

Ecosystem	Aggregate size class	Size(mm)	MRT (yr)
Tropical pasture	M	>200	60
	m	<200	75
Temperate pasture grasses	M	212 – 9500	140
	m	53 – 212	412
Soybean	M	250 – 2000	1.3
	m	100 – 250	7
Corn	M	>250	14
	m	50 – 250	61
Corn	M	>250	42
	m	50 – 250	691
Wheat – fallow, no tillage	M	250 – 2000	27
	m	53 – 250	137
Wheat – fallow, conventional tillage	M	250 – 2000	8
	m	53 – 250	79

Conclusions

Among the carbon isotopic techniques ^{13}C natural abundance is being used in medium term observations (5-60 yrs) whereas oldest and most recalcitrant pools (200-40,000 yrs) can be estimated by bomb ^{14}C analysis. By using isotopic ratio method we can determine the source which contributes to SOM, when there is a transformation in vegetation pattern from C_3 to C_4 or vice-versa. $\Delta^{14}C$ method has been successfully used for partitioning of soil CO_2 efflux and quantifying the contribution of different components of soil respiration. The MRTs of the soil fractions derived from ^{13}C dynamics indicate that the C and N associated with micro-aggregate and silt+clay fractions is physically protected from decay and/or biochemically recalcitrant.

Future aspects

The molecular characteristics of organic matter in organo-mineral associations have to be resolved with knowledge of the isotopic signatures of specific molecules. The potential of combining isotopic analyses of soil respiration rates with other methods (studies of litter bags, mineralization/ immobilization patterns and nutrient uptake in plants) is to be explored.

References

1. Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y. Turnover of soil organic matter and of biomass under C_3-C_4 vegetation change: Consideration of ^{13}C fractionation and preferential substrate utilization. Soil Biology and Biochemistry. 2011; 43:159-166.
2. Boutton TW. Stable carbon isotope ratios of natural materials: II. atmospheric, terrestrial, marine, and freshwater environments, in Coleman, D. C., Fry, B. (eds.): Carbon Isotope Techniques. Academic Press, Inc. San Diego, USA, 1991, 173-185.
3. Brunn S, Six J, Jensen LS, Paustian K. Estimating Turnover of Soil Organic Carbon Fractions based on Radiocarbon Measurements. Radiocarbon. 2005; 47:99-113.
4. Gleixner G, Czimczik CJ, Kramer C, Luhker B, Schmidt MWI. Plant compounds and their turnover and stabilization as soil organic matter. In: Schulze ED *et al* (eds) Global biogeochemical cycles in the climate system. Academic Press, SanDiego, 2001, 201-215.
5. Goh KM. Carbon dating. In *Carbon Isotope Techniques*. Coleman, D.C., Fry, B.(Eds), Academic Press: San Diego, CA, 1991, 125-145.
6. Gude A, Kandeler E, Gleixner G. Input related microbial carbon dynamics of soil organic matter in particle size fractions. Soil Biology and Biochemistry. 2012; 47:209-219.
7. Hardie SM, Garnett MH, Fallick AE, Rowland AP, Ostle NJ, Flowers TH. Abiotic drivers and their interactive effect on the flux and carbon isotope (^{14}C and $\delta^{13}C$) composition of peat-respired CO_2. Soil Biology and Biochemistry. 2011; 43:2432-2440.
8. Lal R. Managing soils for a warming earth in a food-insecure and energy-starved world. Journal of Plant Nutrition and Soil Science. 2010; 173:4-15.
9. Mueller CW, Gutsch M, Kothieringer K, Leifeld J, Rethemeyer J, Brueggemann N *et al*. Bioavailability and isotopic composition of CO_2 released from incubated soil organic matter fractions. Soil Biology and Biochemistry. 2014; 69:168-178.
10. Paul EA. Dynamics of organic matter in soils. Plant and Soil. 1984; 76:275-285.
11. Rodeghiero M, Churkina G, Martinez C, Scholten T, Gianell D, Cescatti A. Components of forest soil CO_2 efflux estimated from $\Delta^{14}C$ values of soil organic matter. Plant and Soil. 2013; 364:55-68.
12. Sanaullah M, Chabbi A, Rumpel C, Kuzyakov Y. Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labeling. Soil Biology and Biochemistry. 2012; 55:132-139.
13. Six J, Jastrow D. Organic matter turnover. *Encyclopedia of Soil Science,* Marcel Dekker Pub, New York, 2002, 936-942.

14. Wolf DC, Legg JO, Boutton TW. Isotopic Methods for the Study of Soil Organic Matter Dynamics. Soil Science Society of America. 1994; 5:865-903.
15. Zhang H, Ding W, Yu H, He X. Carbon uptake by a microbial community during 30-day treatment with ^{13}C-glucose of a sandy loam soil fertilized for 20 years with NPK compost as determined by a GC-C-IRMS analysis of phospholipid fatty acids. Soil Biology and Biochemistry. 2013; 57:228-236.