

Carbon Isotopes in Soil Organic Matter Dynamic Studies

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Abstract

Carbon exists in different isotopic forms *viz.* ^{12}C , ^{13}C and ^{14}C . The degree of carbon fractionation that takes place in a given sample can be estimated by measuring the ratio of amounts of ^{13}C and ^{12}C isotopes, and the ratio ($^{13}\text{C}/^{12}\text{C}$) is expressed as a relative value to the standard *viz.* Pee Dee Belemnite (PDB). ^{13}C is less preferred by soil microbes, as compared to ^{12}C , which results in ^{13}C discrimination in soil. Due to continuous release of more 'light CO_2 ($^{12}\text{CO}_2$)', the evolution of 'heavy CO_2 ($^{13}\text{CO}_2$)' is relatively abridged, resulting in selective enrichment of ^{13}C in the recalcitrant soil organic carbon (SOC) pools. Plants fabricate organic residues with different $^{13}\text{C}/^{12}\text{C}$ composition which could be attributed to their differential ability in utilizing C isotopes. During photosynthetic uptake of CO_2 , C-3 plants discriminate ^{13}C to a higher extent than that of C-4 plants. Thus, relatively lower $\delta^{13}\text{C}$ values is reported in C-3 plants (-22 to -33‰) as compared to higher values in C-4 plants (-9 to -16‰). Reports from the long term fertilizer experiments revealed that $\delta^{13}\text{C}$ value correlated well with deep soil C sequestration. By using $\delta^{13}\text{C}$ value and using empirical equations, the proportion of SOC derived from new and old carbon stocks can be gauged through the mass balance of C isotopes.

Key words: ^{13}C , carbon dynamics, isotopes, soil organic matter

Introduction

Earth is a dynamic system, wherein carbon cycle, move and partition between different components *viz.* plants, soil, ocean, air and even rocks. Plants capture carbon dioxide (CO_2) from the atmosphere in presence of sunlight to make their own food and accumulate as plant biomass, which in turn became animal biomass through food chain system. After death, the plant and animal biomass get decomposed to form soil organic matter. Carbon is the chief constituents in biological compounds as well as a major component of many minerals such as limestone. Meanwhile a part of the carbon will be cycled back through respiration and methane emission. Carbon dioxide from the atmosphere dissolves into water bodies (ocean, lakes, ponds *etc.*). Thus, carbon will be cycled between biosphere, pedosphere, geosphere, hydrosphere, and atmosphere of the Earth. Soil organic carbon (SOC) in agro-ecosystems plays key role in soil fertility, nutrient cycling, sustainability of land through its effect on soil physical, chemical and biological properties (Kabiri *et al.*, 2015; Tian *et al.*, 2015). Soil organic matter (SOM) play vital role in improving the soil resilience and decreases soil erosion (Majumder *et al.*, 2008). Sustainability of

land is harmfully affected by faulty management practises (Qin *et al.*, 2015). Soil organic carbon (SOC) contents are being constantly dwindling and SOC loss is amplified in degraded lands (Zuazo & Pleguezuelo, 2008).

Carbon exists in different isotopic forms *viz.* ^{12}C , ^{13}C and ^{14}C and can be used as tracer to profile various ecological functions and plant adaptations (Raj *et al.*, 2019; Raj *et al.*, 2020). Carbon-12 (^{12}C) and carbon-13 (^{13}C) are stable non radioactive isotopes, and carbon-14 (^{14}C ; also known as radiocarbon) is an unstable radioactive isotope. It was reported that ratio of ^{14}C to ^{12}C is approximately 1.25 parts of ^{14}C to 10^{12} parts of ^{12}C (Tsipenyuk, 1997).

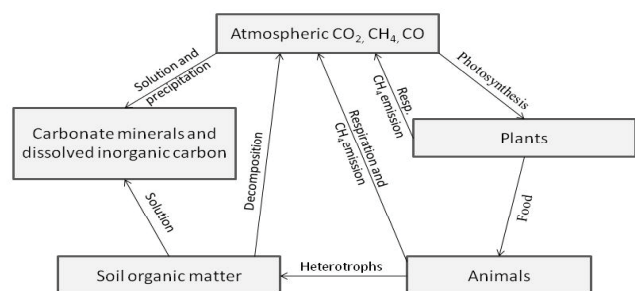


Fig. 1: Cycling of carbon between different components of the earth system

Determining the age of organic materials through radiocarbon dating

The method was developed by Willard Libby. The interaction of cosmic rays with atmospheric nitrogen results in the production of radiocarbon (^{14}C), which combines with oxygen to form radioactive carbon dioxide ($^{14}\text{CO}_2$). Plant incorporates it into the biomass through photosynthesis; animals acquire ^{14}C by consuming the plants. Once the plant or animal dies, uptake of carbon with the environment gets curtailed, and subsequently ^{14}C content begins to decline within the sample through radioactive decay. Assessing the amount of ^{14}C in a dead plant or animal sample provides information that can be used to determine the age of the sample. The older a sample, the lesser will be ^{14}C content in it. Since the half-life of ^{14}C is about 5,730 years, the oldest age that can be consistently measured by this process is about 50,000 years. Further, corrections should be made to account the proportion of ^{14}C fractionation in different types of organisms, and the reservoir effects through varying levels of ^{14}C within the biosphere.

^{13}C discrimination in soils

Carbon discrimination refers to selective accumulation of ^{13}C in SOC. Although ^{13}C discrimination in soils is well recognized, the connection of ^{13}C abundance is not established. Further, it remains ambiguous to clearly trace the effect of such carbon discrimination on the overall distribution of ^{13}C in SOC. ^{13}C was less preferred by soil microbes, as compared to ^{12}C , at early stages of residue decomposition, which would result in ^{13}C discrimination and preferential release of "light CO_2 ($^{12}\text{CO}_2$)" in gaseous form was reported (Flessa et al., 2000). Due to the continuous release of more light CO_2 , "heavy CO_2 ($^{13}\text{CO}_2$)" evolution was relatively abridged. Thus, higher ^{13}C accumulation in soil was the result of discrimination in heavy CO_2 evolution (Dalal et al., 2013).

Carbon sequestration and ^{13}C natural abundance

Carbon sequestration refers to capturing and storing C in long lived pools and it is considered as an effective

strategy to combat land degradation and climate change (Lal, 2004). The quality and quantity of SOC could be improved by annual addition of organic matter (Bhattacharyya et al., 2011). Labile C pools change very frequently with the soil and crop management practises; however recalcitrant SOC is protected within aggregates by long term management practices and eventually add to SOC sequestration (Lenka et al., 2012). Soil aggregation trim down land degradation by shielding SOC and improving C sequestration in agro-ecosystems (Bhattacharyya et al., 2013). The relative proportions of labile pools of SOC (water soluble C, microbial biomass C, and $\text{KMnO}_4\text{-C}$ etc.) are very sensitive to management practices and suggest the suitability of management practice. Conversely, recalcitrant C is protected within the soil aggregates and consequently accounts for SOC sequestration. The information available on soil aggregate characterization is very scanty with respect to the labile and recalcitrant C pools, and the relative abundances of ^{13}C within the soil aggregates (Kocyigit & Demirci, 2012; Six & Paustian, 2014; Yu et al., 2015). Further, the effect of different C pools, C sequestration rates and $\delta^{13}\text{C}$ with long-term crop productivity are inadequate.

Assessment of $\delta^{13}\text{C}$ values in different samples

The carbon isotope ratio refers to the ratio of the amounts ^{13}C to that of ^{12}C present in the sample, expressed relative to a standard known as Pee Dee Belemnite (PDB) expressed in ‰ (Cheng et al., 2011).

$$\delta^{13}\text{C} (\text{‰}) = \left[\frac{\left(\frac{X^h}{X^l} \right)_{\text{sample}}}{\left(\frac{X^h}{X^l} \right)_{\text{standard}}} - 1 \right] \times 1000$$

Where X is carbon, h: heavier C isotope (^{13}C), and l: lighter C isotope (^{12}C). The CO_2 samples must be analysed relative to the internal working gas standards. The carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) are expressed as a relative values to the PDB.

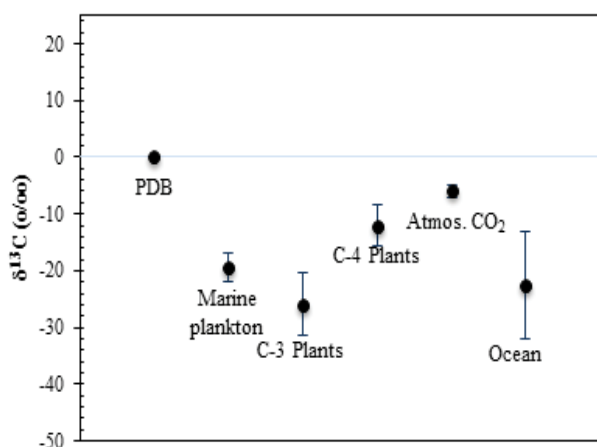


Fig. 2: The isotopic composition of different samples. Pee Dee Belemnite (PDB) is the reference standard. Error bars represents the reported range of δ13C (o/oo)

Source: Deines, 1980; Vitorello et al., 1989; Maslin and Swann, 2006.

Assessing the proportion of C derived from new residue

With help of δ¹³C values of the SOM, it is possible to calculate the proportion of carbon derived from new residues (Balesdent and Mariotti, 1996).

$$f_{new} = \frac{\delta_{new} - \delta_{old}}{\delta_{veg} - \delta_{old}}$$

Table 1: Approaches to study soil organic matter dynamics

Sl. No.	Particulars of the study	Conclusion	Reference
1.	Link between physical soil architectural traits and organic carbon decomposition.	The functional relationship between soil physical properties with the rate of soil organic carbon decomposition within the aggregates was reported.	Li et al., 2016; Rabbi et al., 2016
2.	Turnover of organic matter in soil physical fractions during invasion of woody plant in grassland: evidence from natural ¹³ C and ¹⁵ N.	Higher rate of mineralization was observed in SOM associated with macro aggregate.	Liao et al., 2006
3.	C isotope analyses to assess alteration of chemically separated soil organic carbon pools under long-term fertilization.	Changes in accumulation due to shifts in crop species can be more evident in light fraction of soil organic matter.	Dou et al., 2016

Where δ_{new} represents the δ¹³C values of organic C in soil fractions after a period of time, δ_{old} represents the δ¹³C values of organic C in the initial soil, i.e., the soil samples prior to tillage, and δ_{veg} represents the δ¹³C values of the mixed samples, including plant materials and manure. Further, since we measure δ_{veg}, δ_{new} and δ_{old} independently, the standard errors associated with the proportion estimate (f) can be calculated through a mass-balance approach using partial derivatives (Phillips and Gregg, 2001).

$$\sigma^2 f = \left(\frac{\delta f}{\partial \delta_{veg}}\right)^2 \sigma^2 \delta_{veg} + \left(\frac{\delta f}{\partial \delta_{new}}\right)^2 \sigma^2 \delta_{new} + \left(\frac{\delta f}{\partial \delta_{old}}\right)^2 \sigma^2 \delta_{old}$$

The equation can be rearranged and reduced as;

$$\sigma^2 f = \frac{1}{(\delta_{new} - \delta_{old})^2} [\sigma^2 \delta_{veg} + f^2 \sigma^2 \delta_{new} + (1 - f)^2 \sigma^2 \delta_{old}]$$

Where $\sigma^2 \delta_{veg}$, $\sigma^2 \delta_{new}$ and $\sigma^2 \delta_{old}$ represent the variances of the mean δ_{veg}, δ_{new} and δ_{old}, respectively. $\sigma^2 f$ represents the variance of the proportion (f) estimate (Dou et al., 2017).

The decay rate constant (k) for the old C present in the soil fractions (C of the organic matter before tillage) was calculated based on Cheng et al. (2011):

$$\ln(f_{old}) = -kt$$

where $f_{old} = (1 - f_{new})$ is the proportion of old C, k is the net relative decay rate constant for old C, and t is the age of the cropping treatments.

4.	Dynamics and turnover of organic carbon and nitrogen in soil through the assessment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ changes under pasture and cropping practices.	Differential accumulation of light and heavy C isotopes by C-3 and C-4 plants.	Dalal et al., 2013
5.	Structural convergence of maize and wheat residue during two-year decomposition under different climatic conditions.	Enrichment of ^{13}C with depth of soil.	Wang et al., 2012
6.	Soil organic carbon sequestration under long-term fertilization in an Inceptisol.	Deep soil C sequestration in soybean-wheat cropping system was positively correlated with $\delta^{13}\text{C}$ value.	Ghosh et al., 2018

The change in $\delta^{13}\text{C}$ values after 25 years of long-term fertilization in maize field

A case study of twenty five years of fertilization experiment in monoculture maize (*Zea mays L.*) on Typic Hapludoll of China reported by Dou et al. (2016) revealed that SOC content in the total organic C and labile carbon pools were significantly higher in MNPK (farmyard manure along with balanced inorganic fertilizers) and SNPK (corn straw residue along with balanced inorganic fertilizers) treated plots and lower in inorganic nitrogen fertilizer (IN) and balanced inorganic fertilizers (NPK) treated soils than the corresponding initial values in the surface soil. This has the implication that long-term addition of manure combined with inorganic fertilizers significantly increased SOC content. Higher soil organic carbon pool was noticed in surface ($22.4 \pm 1.2 \text{ g kg}^{-1}$) and subsurface ($21.2 \pm 0.3 \text{ g kg}^{-1}$) MNPK-treated soils, than that of initial surface ($16.8 \pm 1.4 \text{ g kg}^{-1}$) and subsurface ($14.4 \pm 1.0 \text{ g kg}^{-1}$) soil samples, which gave evidence that SOC storage substantially increased both in the surface and subsurface layer. Higher $\delta^{13}\text{C}$ was noticed in surface (-19.7 ± 0.4

‰) and subsurface ($-19.7 \pm 0.2 \text{ ‰}$) SNPK-treated soils due to a higher contribution of C-4 residues in the soil organic pools, than that of initial surface ($-21.3 \pm 0.9 \text{ ‰}$) and subsurface ($-22.2 \pm 0.6 \text{ ‰}$) soil samples. This was attributed to the root dominated inputs of SOC (root biomass and exudates) and the larger corn roots were dispersed mostly in the 20–30 cm soil layer.

$\delta^{13}\text{C}$ changes under cropping systems

The clear mechanisms that resolve changes in SOC dynamics as a consequence of changes in the quantity and composition of residue inputs is not yet fully understood (Mazzilli et al., 2014; McDaniel et al., 2014). The $\delta^{13}\text{C}$ values varied from lower values in C-3 plants (-22 to -33‰) to higher values in C-4 plants (-9 to -16‰) (Vitarello et al., 1989). Plants fabricate organic residues with different $^{13}\text{C}/^{12}\text{C}$ ratios because of their differences in utilizing C isotopes, for instances $\delta^{13}\text{C}$ for maize residue (C-4 plant) is $\sim -12\text{‰}$ and $\delta^{13}\text{C}$ for soybean residue (C-3 plant) is $\sim -28\text{‰}$ (Dalal et al., 2013; Zhang et al., 2015). The relative contribution of new and old SOC can be gauged through the mass balance of C isotope contents, and thus SOM turnover time can be estimated *in-situ* (Zhang et al., 2015). The intermediate isotopic composition derived from mixed C-3 and C-4 vegetation ($\delta^{13}\text{C} = -18$ to -21‰) permit researchers to concurrently follow the diminution in soil $\delta^{13}\text{C}$ after the introduction of C-3 plants or the enrichment following C-4 plants (Dalal et al., 2013;

Mazzilli et al., 2014). Thus, SOM physical fractionation, together with the natural abundance of stable C isotopes, can be considered as a useful approach for measuring SOM dynamics under long-term cropping systems (Wang et al., 2015). The long-standing theory imply that SOM was composed of inherently stable and chemically distinct compounds, while an emergent view confirmed that SOM accounts a continuum of intermediate and progressively decomposing organic compounds (Lehmann and Kleber, 2015).

The changes in soil physical properties were functionally related to the rate of organic carbon decomposition within aggregates (Li et al., 2016; Rabbi et al., 2016). For instance, macro aggregate associated SOM is more sensitive in response to tillage practices than that of micro aggregates (Kabiri et al., 2015) and hence greater SOM content and higher mineralization rates are frequently associated with macro aggregate fractions (Liao et al., 2006). Although light fraction usually represents a small proportion of total soil C, changes in C storage due to shift in crop species can be more evident in light fraction in contrast to bulk soil (Dou et al., 2016). Physical and chemical stabilization of organic matter occur through intra-aggregate particulate organic matter (iPOM), and mineral-associated organic matter (mSOM) represents the heavy and mineral-associated recalcitrant fractions (Mazzilli et al., 2015).

$\delta^{13}\text{C}$ changes in long-term fertilization and its relation with soil C sequestration rates

Although the ^{13}C natural abundance technique was used to study SOC dynamics, the information available on long-term fertilization effects on soil C sequestration and its relation with $\delta^{13}\text{C}$ is scanty. Significant correlation reported between $\delta^{13}\text{C}$ and the SOC sequestration rate demonstrate that $\delta^{13}\text{C}$ values could well predict the stability/recalcitrance of SOC. It was documented natural ^{13}C became more enriched at greater depths (Wang et al., 2012). The depletion of ^{13}C abundance in the surface layer of soil, frequently gain new crop residues, partially indicate the trend of $\delta^{13}\text{C}$ in atmospheric CO_2 (Ghosh et al., 2018). Conversely, enrichment of ^{13}C in the sub-surface soil, which receive older SOC from surface, possibly will be

due to isotopic fractionation during SOC decomposition (Wynn et al., 2005) and lower sensitivity of sub-soil SOC to crop management practises (Flessa et al., 2000). Depletion of ^{13}C in the surface soils of plots receiving no fertilizer or manure input could add less C inputs from plant residues than the plots receiving combined application of NPK fertilizer and manure. Enrichment of ^{13}C in the NPK fertilizer and manure treated plots in all soil layers might be due to their affluence in labile compounds (sugars and cellulose), which are rich in ^{13}C , as compared to lignin and lipids (Hobbie & Werner, 2004). Higher $\delta^{13}\text{C}$ values were also reported in macro aggregates, micro aggregates and bulk soils under NPK fertilizer plus manure treated plots signifies better stability and recalcitrance of SOC. Significant correlation between deep soil C sequestration rates and $\delta^{13}\text{C}$ was reported in soybean-wheat cropping system. NPK fertilizer along with manure application was reported surface and deep soil C sequestration with highest crop productivity (Ghosh et al., 2018). Thus, $\delta^{13}\text{C}$ values are imperative to calculate C stabilization.

Conclusions

Carbon exists in different isotopic forms *viz.* ^{12}C , ^{13}C and ^{14}C and can be used as tracer to profile various ecological functions and plant adaptations. ^{13}C was less preferred by soil microbes, as compared to ^{12}C , which results in ^{13}C discrimination in soil. Assessing the amount of ^{14}C content in a dead plant or animal sample provides information that can be used to determine the age of the sample. The relative contribution of new and old SOC in cropping practice can be gauged through mass balance of C isotope contents. SOM physical fractionation, together with the natural abundance of stable C isotopes, can be considered as a useful approach to measure SOM dynamics under long-term cropping systems. Significant positive correlation reported between $\delta^{13}\text{C}$ and the SOC sequestration implies that $\delta^{13}\text{C}$ values can be used to assess the stability and recalcitrance of SOC.

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