UNIT 11 ISOTOPIC STUDIES FOR ASSESSING ORGANIC MATTER TURNOVER IN SOIL

Structure

11.1 INTRODUCTION

The element carbon has seven isotope forms (^{10}C, ^{11}C, ^{12}C, ^{13}C, ^{14}C, ^{15}C, ^{16}C), among them two are stable (^{12}C and ^{13}C) and five are radioactive (^{10}C, ^{11}C, ^{14}C, ^{15}C, ^{16}C) with half-life times varying between 0.74 seconds (^{16}C) and 5,726 years (^{14}C) (Holmen, 2000). The natural abundances of ^{12}C, ^{13}C and ^{14}C isotopes are about 99.9% of the total carbon present in our environment. The most common form of carbon isotope is carbon-12 (^{12}C), which has 6 neutrons and 6 protons in its nucleus. The second form of carbon isotope is carbon-13 (^{13}C), which has 7 neutrons. The third form of carbon isotope is carbon-14 (^{14}C), which has eight neutrons in its nucleus. The ^{14}C is radioactive (unstable) and decays by emission of an electron to form ^{14}N with a half-life of 5,726 years

(West, 2008). The ^{14}C make up $<10^{-12}$% of carbon in our environment (Staddon, 2004). The ^{12}C and ^{13}C isotopes are called as stable carbon isotopes because they are not radioactive. The natural abundance of the ^{12}C and ^{13}C isotopes of total carbon is 98.982% and 1.108% respectively (Staddon, 2004).

11.2 OBJECTIVES

After studying this unit, you should be able to:

- write about stable carbon isotopes;
- explain stable carbon isotopes enrichment;
- describe the use of ^{13}C isotopes in soil organic matter turnover and carbon stabilization studies;
- explain the d*etermination of* $\delta^{13}C$ by *isotope ratio mass spectrometer (*IRMS); and
- explain the detection of ^{13}C by NMR spectroscopy.

11.3 STABLE CARBON ISOTOPES

The ratios of ^{13}C and ^{12}C isotopes ($\delta^{13}C$) are commonly expressed in parts per thousand, per mil (‰), relative to an international carbonate standard (Vienna Pee Dee Belemnite, VPDB). The isotopic ratios (^{13}C and ^{12}C) of VPDB is 0.00112372. The $\delta^{13}C$ can be derived from the following universal equation.

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

Where the $R_{sample} - R_{standard}$ is ^{13}C:^{12}C ratios of the sample and reference standard (V-PDB), respectively. The R represents the mass, 45/44, ratio of the sample or standard (Amelung et al., 2008). The negative $\delta^{13}C$ values indicate that ^{13}C is less abundant than in the reference standard (see more detail in the section determination of $\delta^{13}C$). For example, if a sample contains -25‰ then the $^{13}C/^{12}C$ ratio of the sample is 2.5% or 25 parts per thousand lower than the VPDB standard. Figure 11.1 shows the average $\delta^{13}C$ values of atmosphere, vegetation, soil and soil biota. After the industrial revolution, the $\delta^{13}C$ value of atmospheric CO_2 has increased from -6.4‰ to -8.0‰ (O'Leary, 1981; Staddon, 2004). The shift in the value of $\delta^{13}C$ (more negative) in the atmosphere is due to the combustion of more fossil fuels and land use change.

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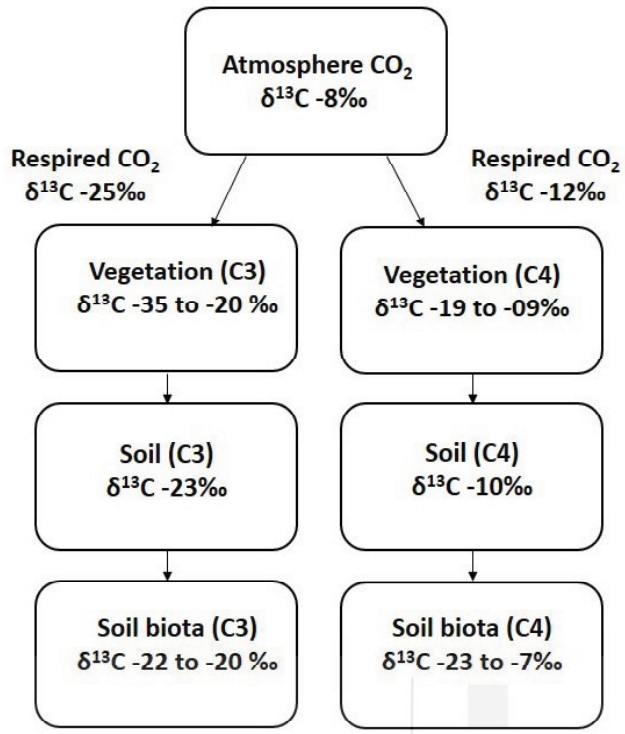

Fig. 11.1: $\delta^{13}C$ values in C3 and C4 dominated ecosystems of the planet earth. (Adapted from Staddon 2004; Boutton, 1991).

Besides, mostly plant-derived organic matter and metabolites are ^{13}C depleted with the standard VPDB, because of isotopic fractionation during photosynthesis (O'Leary, 1981). The largest part of isotopic fractionation occurred during the production of organic matter by primary producers (plants and photosynthetic bacteria).

11.4 CARBON ISOTOPIC FRACTIONATION DURING PHOTOSYNTHESIS

The carbon isotopic fractionation occurred in several steps during the process of photosynthesis by plants (Park and Epstein, 1960; O'Leary, 1981). For example, the carbon isotopic fractionation is taking place at each step of CO_2 uptake viz., diffusion, dissolution, carboxylation, and respiration by different types of plants in various environmental conditions (O'Leary, 1981). Table 11.1 shows the carbon isotopic fractionation occurred during the different metabolic process of photosynthesis. Several models assumed that the overall carbon isotopic fractionation occurred during photosynthesis is entirely due to differences in $^{12}C/^{13}C$ contents of CO_2 across the stomata pathway and the fractionation by Ribulose-1,5-bisphosphate carboxylase (Rubisco) (Farquhar, 1989). The difference in isotopic compositions in the metabolic compounds, derives from the biochemical process (photosynthesis), is due to the enzymatic isotopic effect (Tcherkez et al, 2011). Therefore, the photosynthetic fractionating of carbon isotopes (products at each step of photosynthesis process gets ^{13}C enriched) is termed as a kinetic process (White, 2015).

Table 11.1: Carbon isotopic fractionation during photosynthesis (adapted from O'Leary, 1988; Farquhar, 1989). Note: Positive values indicates ^{13}C value in the product is depleted as compared to starting state and negative values indicate enrichment.

Process	Isotopic fractionation Δδ*, ‰	Reference
Diffusion of CO_2 from atmospheric air to stomatal pore	4.4	Craig, 1954; O'Leary, 1981
Diffusion of CO_2 from the leaf boundary layer to stomatal pore	2.9	Farquhar, 1983
Diffusion of CO_2 in water	0.7	O'Leary, 1984
Phosphoenolpyruvate carboxylase catalyzed a reaction of HCO_3- with phosphoenol pyruvate	2.0	O'Leary et al., 1981
Ribulose bisphosphate carboxylase catalyzed a reaction of CO_2 with ribulose bisphosphate	29	Roeske and O'Leary, 1984
Fixation of gaseous CO_2 (in equilibrium with HCO_3 at 25°C) by PEP carboxylase	-5.7	Farquhar, 1983
Equilibrium hydration of CO_2 at 25°C	-9.0	Mook et al., 1974

Δδ* indicates the difference in values

Plants take carbon in the form of carbon dioxide (^{12}C, ^{13}C and ^{14}C) during photosynthesis mostly by two different mechanisms, which are named the C_3 and C_4 pathway depending on whether CO_2 is initially coupled to a 3-C or a 4-C compound (Smith and Brown, 1973; Farquhar, 1989; Pyankov et al., 2000). C3 plants are the plants that produce a three-carbon compound as the first stable product during photosynthesis. Examples of C3 plants are rice, wheat, etc. On the other hand, C4 plants produce a four-carbon compound as the first stable product during photosynthesis. Examples of C4 plants are maize, sugar cane, etc. About 85% of plants operate C3 type of photosynthesis, where CO_2 is fixed by ribulose-bisphosphate carboxylase and produce two molecules of phosphoglyceric acid. Thus, carbon fixed by C3 plants assimilate more ^{12}C (lighter isotope) as compared to ^{13}C (heavier isotope), and as a result of this isotopic fractionation, the tissues of plants have lower $^{12}C/^{13}C$ ratios than the atmospheric CO_2 (Figure 11.1). As a result, plants with the C3 (Calvin cycle) pathway have $\delta^{13}C$ values between -35‰ and -20‰ (Boutton, 1991). Likewise, in C4 photosynthesis, the initial fixation of CO_2 in mesophyll cells by phosphoenol pyruvate carboxylase and subsequently to produce oxaloacetate. Plants with the C4 (Hatch-Slack) pathway have higher $\delta^{13}C$ values ranging from -19‰ to -9‰. In addition, plants with crassulacean acid metabolism (CAM) pathways have $\delta^{13}C$ values ranging from -17‰ to -9‰ (Accoe et al., 2002). Generally,

the C3 plants discriminate more against ^{13}C than C4 plants during the photosynthetic uptake of CO_2 (Farquhar et al., 1989). Plants used to have less ^{13}C than the atmospheric CO_2 because of the physical and chemical process involved in the uptake of CO_2 and discrimination against ^{13}C (O'Leary, 1988). As we know that ^{13}C isotope is heavier than ^{12}C isotope and thus during diffusion process the $^{13}CO_2$ diffuse slower than that of $^{12}CO_2$. More specifically, an earlier study (O'Leary, 1988) defined the process of carbon isotopic fractionation (from one metabolic product to other metabolic product) in the following equation model.

Isotopic fractionation $(\Delta\delta) = [\delta^{13}C(A) - \delta^{13}C(B)] / 1 + \delta^{13}C(A) / 1000$

Where the A and B are the $\delta^{13}C$ values of product A and B respectively. The isotopic fractionation unit is ‰. Thus, one can calculate the differences in an isotopic fractionation ($\Delta\delta$) of the initial and final metabolic products derived during the process of photosynthesis.

11.5 STABLE CARBON ISOTOPES IN SOILS

Mostly, we expect the differences in $\delta^{13}C$ values of soils and vegetation, even in the absence of C3 or C4 vegetation conversion, in the stabilized ecosystem. Several studies have been observed the progressive enrichment of $\delta^{13}C$ values in soils with increasing depth under C3 vegetation (Ehleringer et al., 2000; Mehta et al., 2012; Dinakaran et al., 2018). Therefore, the difference in $\delta^{13}C$ values of soil and plant biomass (litter) is called discrimination factor (DFi) and it can be calculated by the following equation (Garten et al., 2000).

$$DFi = \delta^{13}Csoil - \delta^{13}C \text{ litter input}$$

Where the $\delta^{13}Csoil$ and $\delta^{13}C$ litter input represents the $\delta^{13}C$ values of surface soil and $\delta^{13}C$ values of leaf litter respectively. Thus the discrimination factor value can tell whether the organic matter in the soil is derived from the C3 or C4 vegetation. For example, the C4 vegetation would be identified by a larger discrimination factor (9-10‰) values in a natural ecosystem (Martinelli et al., 1996). Whereas if the discrimination factor values ranges from 2‰ to 7‰, then it could be attributed to isotopic fractionation associated with SOM decomposition (Ehleringer et al., 2000; Dinakaran and Krishnayya, 2010).

11.6 STABLE CARBON ISOTOPES ($\delta^{13}C$) ENRICHMENT HYPOTHESES

The progressive enrichment of $\delta^{13}C$ in the soil profiles under C3 dominated ecosystem is related to humification (humus formation) process (Ehleringer et al., 2000). Several studies have observed the enrichment of $\delta^{13}C$ values with increasing soil depths under C3 vegetation cover (Ehleringer et al., 2000; Mehta et al., 2013; Wang et al., 2015; Dinakaran et al., 2018). The SOM in deeper soil profiles (>50cm) is considered as old and highly decomposed in nature (Trumbore, 2000). Thus the progressive enrichment of $\delta^{13}C$ from surface soil layers to deeper layers under the C3 vegetation community may be useful in calculating the turnover rates of carbon (Wang and Hsieh, 2002). Although there is a debate over the changes in $\delta^{13}C$ of SOM and factors controlling the process in the same soil profiles under C3 vegetation community. There have been four different hypotheses available in

the literature related to $\delta^{13}C$ enrichment in the soil profiles (Ehleringer et al., 2000). They are 1) Suess effect, 2) Microbial fractionation during litter decomposition 3) preferential utilization of substrates in organic matter by microbes and 4) soil carbon mixing.

11.6.1 Suess Effect

The emission of CO_2 from the combustion of fossil fuels (i.e. depleted in ^{13}C) and land use change decreases the $^{13}C/^{12}C$ ratio of atmospheric CO_2 is called a Suess effect (Keeling, 1979). Earlier studies have noticed a very small difference (-1.7‰) in the values of $\delta^{13}C$ in the atmosphere during the year between 1774 and 1993 (Freidli et al., 1987; Trolier et al., 1996). Whereas the average discrimination factor (DFi) of C3 dominated forest ecosystem is -3.0‰ (Ehleringer et al., 2000; Powers and Schlesinger, 2002). Thus the larger discrimination factor values (>-1.7‰) in any ecosystem are not due to the Suess effect. Moreover, several studies have proved that the organic matter in deeper soil layers is highly humified and much older than surface soil organic matter (Trumbore, 2000; Eusterhues et al., 2003; Dinakaran et al., 2018). By using the differences in $\delta^{13}C$ values of atmosphere, litter, soil; one can estimate the turnover rates of carbon if other environmental factors are not influencing the ^{13}C enrichment of soils in the particular ecosystem. Therefore, the larger difference of $\delta^{13}C$ values (>-1.7‰), from surface litter to soil layers, in the stabilized C3 ecosystem is entirely due to SOM decomposition by microbes.

11.6.2 Microbial Fractionation during Litter Decomposition

Microorganisms are playing a pivotal role in the decomposition of organic matter in soils. If microbes utilize the ^{13}C depleted substrates from the organic matter during the process of decomposition, then the residual organic matter in the soil would be more positive in $\delta^{13}C$ values (Ehleringer et al., 2000). More specifically, the loss of more ^{12}C enriched CO_2 during the SOM decomposition by microbes. The Rayleigh distillation process is better to describe the gradual enrichment of $\delta^{13}C$ in soil resulting from isotopic fractionation associated with organic matter decomposition process (Accoe et al., 2002). It can be better explained by the following equation.

$$\delta = \delta_0 + \varepsilon \ln[C/C_0]$$

where δ_0 and C_0 represents the initial $\delta^{13}C$ signatures and initial carbon concentrations (surface layers) of the reference system. Besides, the relationship between total carbon contents and their corresponding $\delta^{13}C$ signatures could be better fitted by the Rayleigh equation (Accoe et al., 2002). The slope (ε-fractionation factor) of a linear regression relating isotopic composition ($\delta^{13}C$) to the logarithm of carbon concentration to describe isotopic fractionation associated with SOM decomposition by microbes (Garten et al., 2000). Thus, the larger ε value means a greater isotopic fractionation associated with SOM decomposition (mineralization). It can be simply defined by the following equation (Wang et al., 2015).

$$\text{Fractionation factor } (\varepsilon) = R_{SOM} / R_{CO2}$$

where R_{SOM} and R_{CO2} are the $\delta^{13}C$ values of organic carbon (substrate) and respired CO_2 respectively. Generally, the CO_2 emitted via soil respiration contains comparatively more ^{12}C than ^{13}C in the soil. If your logarithmic carbon contents

and $\delta^{13}C$ values are not linearly related then some other belowground factors (substrates, microbes, and fine roots) may affect the ^{13}C enrichment of soils.

11.6.3 Preferential Utilization of Substrates in Soils by Microbes

The $^{13}C/^{12}C$ ratios of plant metabolic compounds (from the process of photosynthesis) vary from 1.9‰ to 10.3‰ (Hobbie and Werner, 2004). Therefore, if microbes utilize the specific substrates from the organic matter in the soil during the mineralization process, then it must reflect in the $^{13}C/^{12}C$ ratios of the remaining organic matter in the soil. The easily available compounds (sugar and cellulose) for the utilization by microbes are ^{13}C enriched as compared to the less available compounds such as lipids and lignin in natural ecosystems (Hobbie and Werner, 2004; Blagodatskaya et al., 2011). The continuous addition of substrates (roots and leaf litter) into the soil and the substrates utilization pattern of microbes (bacteria and fungi) play a greater role in the shift of $\delta^{13}C$ in the soils (Rinnan and Baath, 2009). Several studies have agreed that lignin and lipids (stable and ^{13}C depleted) have slowly accumulated in the soil during the initial stages of litter decomposition (Hobbie and Werner, 2004; Rinnan and Baath, 2009; Wynn et al., 2006). Other factors such as the addition of biomass from microbes and their by-products during the humification process could have an adverse effect on ^{13}C enrichment in the soils (Wynn et al., 2006). The $\delta^{13}C$ values of lignin compounds are lighter than bulk litter (O'Leary, 1981) and the trend towards increasing concentration of lignin in residual SOM (^{13}C enriched) at deeper soil layers is contradictory to this hypothesis.

11.6.4 Soil Carbon Mixing

Several studies have suggested that the shifts in the utilization of old and new organic matter in soils by microbes during decomposition could shift the $\delta^{13}C$ values (Ehleringer et al., 2000; Dinakaran et al., 2018). Earlier studies have observed that the microbes are ^{13}C enriched than their substrates (Staddon, 2004; Blagodatskaya et al., 2007). Generally, after the humification process, the SOM enriched with microbial-derived compounds and the residual SOM would be $\delta^{13}C$ enriched (Ehleringer et al., 2000). In soils, the *r*-strategists (microbes) grow very rapidly on easily available substrates, whereas the *k*-strategists grow very slowly and utilize the substrates (lignin derivatives) very efficiently (Fierer et al., 2007; Trivedi et al., 2013). Thus, in natural ecosystems, two different types of microbes (*r* and *k* strategists) play a greater role in utilization of substrates in the soils. Therefore, the amount of easily available substrates (from fine roots) in any type of soil ecosystem determines the abundance of *r* and *k* strategists of microbes (Blagodatskaya et al., 2007). Therefore, many researchers believe that the ^{13}C enrichment in soils under C3 vegetation community could be due to the incorporation of microbial-derived products to the residual SOM and Suess effect.

Check Your Progress 1

Note: i) Use the space given below for your answers.
ii) Check your answers with those given at the end of the unit.

1) What are C3 plants?

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2) What are C4 plants?

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11.7 STABLE CARBON ISOTOPES IN SOIL ORGANIC MATTER (SOM)

The stable carbon isotope ($\delta^{13}C$) values in soil organic matter (SOM) are comparable to the source plant litter viz., leaves, twigs, flowers, barks, and died plants (Ehleringer et al., 2000; Wang and Hsieh, 2002), because the amounts of ^{12}C and ^{13}C in the dead litter remain the same (i.e. stable isotopes) in the soil for a longer period. Besides, the isotopic composition of SOM closely resembles the isotopic composition of the vegetation carbon from (C3, C4, and CAM plants) which it has been derived (Powers and Schlesinger, 2002; Wang et al., 2015; Dinakaran et al., 2018). If there is a major shift from C3 to C4 vegetation community in any forest ecosystem by anthropogenic activity, then it would affect the changes in belowground inputs from the newly dominated plant community (agricultural or grassland) in the particular ecosystem. Accordingly, the newly dominated plant community would leave their $\delta^{13}C$ signatures in the SOM. Thus, the use of $\delta^{13}C$ of SOM enables a distinction between separate organic matter pools derived from the original and new vegetation, respectively, as long as one of the vegetation communities (original or new) is predominantly of the C3 and the other of the C4 type (Boutton et al., 1998; Bernoux et al., 1998). Therefore, the stable isotope technique will give us more insight into the SOM dynamics associated with changes in vegetation cover (C3 to C4 and vice versa) and land use change (Oelbermann et al., 2006).

11.8 USE OF ^{13}C ISOTOPES IN SOIL ORGANIC MATTER TURNOVER AND CARBON STABILIZATION STUDIES

The change in land use and cover in any ecosystem (forest to pasture or forest to cultivated or pasture to cultivated or cultivated to pasture) have changed the plant communities (C3 to C4 or vice versa) above and belowground inputs to the soils. In the previous section, we have seen the differences in $^{13}C/^{12}C$ signatures of litter and soils from different plant communities. The organic matter in the soil would have $^{13}C/^{12}C$ signatures from the new plant communities as

well as from the old plant communities. There are three different mathematical approaches available in the literature to estimate the SOM turnover in the natural ecosystem (Bernoux et al., 1998). The first approach is a simple two-compartment model, where a change in land use changed the plant litter inputs (C3 to C4 or C4 to C3) into the soil and it can be used to estimate the proportion (Ft) of organic matter/ organic carbon in the soil derived from new plant communities and old plant communities. At a given time (*t*), the plant community A can be replaced by plant community B and it can be detected in the soil $\delta^{13}C$ values. If the initial and /or old (A) soil $\delta^{13}C$ and final or new (B) soil $\delta^{13}C$ are known, then we can calculate the δt and Ft by the following equation models

$$\delta t = Ft \times \delta B + (1- Ft) \times \delta A$$

$$Ft = (\delta t - \delta A) / (\delta B - \delta A)$$

Several researchers have assumed that the δA equal to the soil $\delta^{13}C$ of a reference site in a similar soil type, climate, and topography where the land use and cover had changed i.e δB. Besides, few more researchers (Desjardins et al., 1994) have used the $\delta^{13}C$ values of the selected plant parts like leaves, roots and the litter of new plant communities (δB). More importantly, the changes in $\delta^{13}C$ values of litter to SOM cannot be used as the reference to plant community change in any C3 dominated ecosystem (Bernoux et al., 1998). If the Ft is calculated, the new carbon pool (size) derived from the new plant communities (CA_t) can be calculated from the old plant communities (CB_t) by the following equation model (Bernoux et al., 1998).

$$CA_t = (1 - Ft) \times CT_t$$

$$CB_t = Ft \times CT_t$$

Care must be taken when you calculate the soil carbon stock (CT) by using the soil bulk density, because it may affect the change in the new carbon stock values. Thus the same approach can be used to calculate the SOM turnover of the particular forest ecosystem. For example, one can calculate the carbon derived from the C3 and C4 vegetation in the converted land using $\delta^{13}C$ of aboveground litter, soil and roots in the stabilized ecosystem by the following equation (Balesdent et al. 1987; Rhoades et al., 2000).

$$\text{C3 derived carbon} = (\delta^{13}C_{CLS} - \delta^{13}C_{4LI}) / (\delta^{13}C_{FS} - \delta^{13}C_{4LI})$$

$$\text{C4 derived carbon} = (\delta^{13}C_{CLS} - \delta^{13}C_{FS}) / (\delta^{13}C_{4LI} - \delta^{13}C_{FS})$$

where $\delta^{13}C_{CLS}$ is the $\delta^{13}C$ value of converted land soil (C4); $\delta^{13}C_{4LI}$ is the C4 litter inputs; $\delta^{13}C_{FS}$ is the $\delta^{13}C$ value of original forest soil. Thus, one can calculate the turnover rates of carbon by using the difference in $\delta^{13}C$ values of SOM from old and recent plant communities occupied in a particular region. Once you calculate the new carbon (F) in the SOM from the recent plant communities, the mean residence time (MRT) can be calculated based on the old fraction of carbon under steady-state conditions (Amelung et al., 2008).

$$MRT = -T / (1-F)$$

where T is time.

The estimated turnover rates of SOM by the natural abundance of $\delta^{13}C$ and artificial labelling techniques are entirely based on the changes of SOM occurred

during the vegetation shift over the decade period. Besides, it is possible to manipulate the natural abundance of $\delta^{13}C$ values by adding artificial labelling substrates in the soil to estimate the turnover of carbon.

11.9 ARTIFICIAL LABELLING TECHNIQUE TO ESTIMATE SOM TURNOVER

The most common and well established artificial labelling technique is called free air CO_2 enrichment method (FACE). By this method, one can increase the concentration of CO_2 above the current atmospheric CO_2 in controlled field conditions. The artificial CO_2 exhibits differences in the values $\delta^{13}C$ (-25‰ to -70‰) as compared to the atmospheric CO_2 (-8‰) (Amelung et al., 2008). Thus the plants grown in the FACE experiments have been labelled with ^{13}C artificially as compared to plants grown in ambient conditions. The new carbon fractions (F) from the artificial CO_2 source in the existing carbon pools can be estimated by the following simple equation (Amelung et al., 2008).

$$F = (\delta_{final} - \delta_{initial}) / (\delta_{source} - \delta_{initial})$$

where F is the fraction of carbon in the soil from the artificial labelling, δ_{source} is the $\delta^{13}C$ of the CO_2 / source carbon applied to the soil, $\delta_{initial}$ and δ_{final} represents the $\delta^{13}C$ of the SOM at the beginning and the end of the experimental period respectively. Till now, the FACE with ^{13}C labelled experiments are laboratory-based and/or rely on small field experimental conditions for a shorter period of time (Blagodatskaya et al., 2011).

11.10 DETERMINATION OF $\delta^{13}C$ BY ISOTOPE RATIO MASS SPECTROMETER (IRMS)

There are two most common isotope ratio mass spectrometer (IRMS) been used to determine the $\delta^{13}C$ in bulk as well as specific compound samples. They are 1) Elemental analyzer coupled with IRMS (EA-IRMS) and 2) Gas chromatography coupled to a combustion furnace and IRMS (GC-C-IRMS). Thus the $^{13}C/^{12}C$ (ratio of carbon 13 and carbon 12) in the litter, soil, and microorganisms would be determined by isotope ratio mass spectrometer (IRMS). The Vienna-Pee-Dee Belemnite (VPDB) is the main standard or reference for determining carbon 13 isotopic contents in natural samples. The reference material was from a Cretaceous fossil, Belemnitella americana from a Pee Dee formation in South Carolina, USA. The isotopic ratio ($^{13}C/^{12}C$) of VPDB is 0.0112372.

11.10.1 The working principle of IRMS (GC-IRMS)

First, the solid sample (litter, soil, sediments, and carbonates) is converted into a gaseous form, for example, CO_2 by the combustion process. After that, the combustion products transferred into the mass spectrometer, where they are ionized, accelerated and separated according to their specific mass-to-charge ratio and these ions are detected by the Faraday cups (for carbon the masses are 44, 45 and 46). The gas chromatography coupled with IRMS is an advanced and specialized instrument to measure the $^{13}C/^{12}C$ ratio in the compound-specific samples.

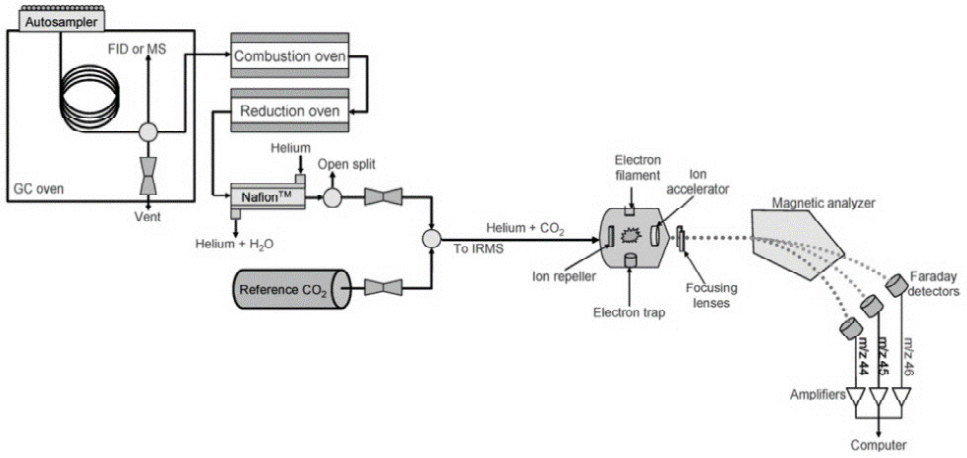

Fig 11.2: The schematic diagram shows how samples are introduced into the systems for carbon isotope measurements (as CO_2) in gas chromatography coupled with isotope ratio mass spectrometer, GC-IRMS (adapted from Muccio and Jackson, 2009).

11.11 DETECTION OF ^{13}C BY NMR SPECTROSCOPY

SOM composed of several chemical compounds and the SOM is mostly derived from plants, microbes, and other faunal communities. The major components of plant tissues are cellulose, hemicellulose, proteins, lignin, and tannins, whereas the microbes and animals are majorly composed of carbohydrates, chitin, peptidoglycan, lipopolysaccharides and lipids (Lorenz et al., 2007). The nuclear magnetic resonance (NMR) spectroscopy has become the most important method for determining the chemical composition of SOM (Preston, 1996). The $^{1}H_{1}$ (proton NMR) and ^{13}C (^{13}C NMR) nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS NMR) technique has been used to determine the chemical composition of organic carbon in litter, soil and humus (Preston, 1997; Lorenz and Preston, 2002; Shiau et al., 2017). The proton NMR technique characterizes the chemical compositions of the organic matter in the soil according to their C-H bonds or number of hydrogen atoms present in the samples, mostly the chemical shift regions in the spectra are ranging from 0 to 12ppm (Dinakaran and Krishnayya, 2010). The proton NMR technique is characterized by the chemical compositions of the organic matter in the samples by following chemical shifts regions in the spectra. They are 1) for alkyl carbon, the chemical shift region in the spectra is 0.5-2 ppm; 2) for O-alkyl carbon, the region is 3-5 ppm in the spectra; 3) for the aromatic carbon, the region would be in 7-8 ppm; and 4) for the carboxyl carbon, the region is 10-12 ppm in the spectra. The ^{13}C NMR technique characterizes the chemical compositions of organic matter based on the number of carbon atoms present in the samples. Table 11.2 shows the ^{13}C NMR regions (spectra) assigned to the specific types of chemical groups.

Table 11.2: Spectra regions and chemical groups acquired from ^{13}C NMR spectrometer (solid state (Adapted from Kinchesh et al., 1995; Smernik et al., 2008).

Spectra region	***Chemical groups / Carbon type***
0-45 ppm	*Alkyl carbon*
45-110 ppm	O-alkyl carbon (methoxyl and carbohydrates derivatives)
110-165 ppm	Aromatic carbon
165-185 ppm	Carboxyl group carbon and derivatives
185-235 ppm	Aryl carbon
235-255 ppm	Carboxyl carbon

The ^{13}C NMR spectroscopy (solution or solid) is extensively used to characterize the chemical compositions of organic matter in the whole soil and particle size fractions. The differences in the relative proportions of the broad functional groups (alkyl, O-alkyl, aromatic, and carboxyl) in the spectra derived from the soil/litter samples could reveal the changes in chemical compositions of organic matter during mineralization. For example, as litter decomposition proceeds, the O-alkyl groups (represents carbohydrates) decreases while the alkyl group (mostly microbial products) increases in the soils (Baldock et al., 1997). The aromatic carbon is assumed to be highly resistant to decomposition and accumulate more in the deeper layers of soils (Baldock et al., 1997; Cepakova and Frouz, 2015). The ^{13}C NMR spectroscopy can be used to determine the chemical compositions of organic matter in the litter, soil and particle size fractions. Therefore, the combination of stable isotopes (δ^{13}) with NMR technique would decipher more information about how organic matter enter into the soil, stabilize and turnover in the soils.

Check Your Progress 2

Note: i) Use the space given below for your answers.
ii) Check your answers with those given at the end of the unit.

1) What is Isotope Ratio Mass Spectrometry?

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2) What is NMR spectroscopy?

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3) What is Soil Organic Matter?

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11.12 LET US SUM UP

The natural abundance of ^{13}C, ^{12}C and ^{14}C isotopes of total carbon is about 99.9% in our environment. The ^{13}C and ^{12}C isotopes are called stable carbon isotopes whereas the ^{14}C is called radioactive isotope. The ratios of ^{13}C:^{12}C ($\delta^{13}C$) are expressed as parts per thousand or per mil (‰). The ^{13}C and ^{12}C isotopes are stable in nature, and they remain in an environment for a longer period. Thus, the stable carbon isotopes ($\delta^{13}C$) are broadly used to identify the paleovegetation history (whether C3 plants or C4 plants) and determine the soil organic matter (SOM) turnover rates of the particular ecosystem. The $\delta^{13}C$ signatures of plants are broadly differed according to their photosynthetic pathways, i.e. C3, C4, and CAM plants. The SOM turnover can be calculated by using the $\delta^{13}C$ values of SOM from old and recent plant communities occupied in a particular ecosystem. Recently, many scientists have manipulated the natural abundance of $\delta^{13}C$ values by adding artificial labelling substrates (^{13}C) in the soil to estimate the turnover rates of carbon. The free air CO_2 enrichment method (FACE) has been widely used to determine the SOM turnover rates in small field conditions. The e*lemental analyzer coupled with isotope ratio mass spectrometer (EA-IRMS) and* gas chromatography coupled to a combustion furnace with IRMS (GC-C-IRMS) have been extensively used to determine the $\delta^{13}C$ *in bulk as well as in the specific compound samples respectively. Besides, the* $^{1}H_{1}$ *(proton NMR) and* ^{13}C *nuclear magnetic resonance spectroscopy (*^{13}C *NMR) have been used to determine the chemical composition of SOM. The combination of stable carbon isotopes (*δ^{13}*) with NMR techniques would open a new view of understating about the organic matter stabilization and turnover in the soils.*

11.13 KEY WORDS

$\delta^{13}C$: Ratios of ^{13}C and ^{12}C in the bulk samples (litter, soil, etc.) and expressed in parts per thousand or per mil (‰).

^{13}C Enrichment : The bulk sample contains more ^{13}C thar ^{12}C isotopes. For example, the stable carbon isotopes ($\delta^{13}C$) values in the soils increase with soil depths under stabilized C3 plant system.

C3 : In C3 plants, during photosynthesis, the CO_2 is initially fixed into 3C compound (3-phosphoglyseric acid, PGA).

C4 : In C4 plants, during photosynthesis, the CO_2 is initially fixed into 4C compound (maleic or aspartic acid).

Carbon Isotopic Fractionation or Discrimination : Alterations in the ratios of ^{13}C and ^{12}C during natural biochemical processes such as photosynthesis, respiration and decomposition.

FACE Experiment : Free air CO_2 enrichment (FACE) is a small scale field experimental setup where one can increase the CO_2 levels without altering the other environmental conditions.

Isotope Ratio Mass Spectrometry (IRMS) : It is a common analytical technique where one can measure the ^{13}C:^{12}C ratios in bulk samples (litter, soil, etc.). The GC-IRMS (Gas chromatography coupled to IRMS) is mostly used to measure the ^{13}C:^{12}C ratios in specific compounds.

NMR Spectroscopy : Nuclear Magnetic Resonance spectroscopy (NMR) is an analytical chemistry technique used to determine the molecular structure of the desired and/or known compounds. The proton ($^1H_1$) and ^{13}C NMR spectroscopy techniques are mostly used to determine the chemical compositions of the SOM.

Soil Organic Matter (SOM) : It is an important component of soil. SOM is mainly derived from plant residues, followed by animals, microorganisms and microbial derivatives at different stages of decomposition. SOM is the store house of nutrients.

SOM Turnover : It is calculated as the elements (carbon, nitrogen and phosphorus) mean residence time (MRT) or its half life time in an environment. In other terms, the time required to decompose the existing SOM stock in a particular ecosystem.

VPDB : Vienna-Pee-Dee Belemnite. VPDB is commonly used standard for determination of $\delta^{13}C$ in samples. This standard was from a Cretaceous fossil from a Pee Dee formation in South Carolina, USA.

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11.15 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

1. C3 plants are plants that produce a three-carbon compound as the first stable product during photosynthesis. E.g: rice, wheat, etc.

2. C4 plants are plants that produce a four-carbon compound as the first stable product during photosynthesis. E.g: maize, sugar cane, millet and sorghum.

Check Your Progress 2

1. Isotope Ratio Mass Spectrometry (IRMS) is a common analytical technique where one can measure the ^{13}C:^{12}C ratios in bulk samples (litter, soil, etc.). The GC-IRMS (Gas chromatography coupled to IRMS) is mostly used to measure the ^{13}C:^{12}C ratios in specific compounds.

2. Nuclear Magnetic Resonance spectroscopy (NMR) is an analytical chemistry technique used to determine the molecular structure of the desired and/ or known compounds. The proton ($^{1}H_{1}$) and ^{13}C NMR spectroscopy techniques are mostly used to determine the chemical compositions of the SOM.

3. Soil organic matter (SOM) is an important component of soil. SOM is mainly derived from plant residues, followed by animals, microorganisms and microbial derivatives at different stages of decomposition. SOM is the store house of nutrients.