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Applications of stable carbon isotopes in soil science with special attention to natural ^{13}C abundance approach

Dóra ZACHÁRY[1,2]

Abstract

Since the invention of the isotope ratio mass spectrometer in the late 1930s, isotope analysis has shed light on many key processes in the Earth's ecosystems. Stable isotope analysis was first applied in the field of chemistry and geochemistry in the 1940s, while the use of isotopic fractionation for various biochemical reactions was elaborated later. The knowledge gained from isotope research led to a better understanding of the dynamics of the biosphere and to the more efficient study of interactions between the geosphere and biosphere. In soil research, stable isotopes are ideally suited to provide a wider insight into the element cycles in soil ecosystems. Stable carbon isotopes, in particular, have been in the focus of soil research, since soil organic matter (SOM) plays an important role not only in soil fertility, soil water management and many other physical, chemical and biological soil functions, but also in the global carbon cycle. If processes connected with these soil functions are isotopically labelled with stable carbon isotopes, the key reactions of C input, exchange and output in the soil and other soil organic matter functions can be studied accurately. The ^{13}C abundance approach is one of the useful methods applying natural stable carbon isotope differences in the atmosphere-plant-soil system to track the stability of organic carbon in these reservoirs. The turnover of SOM, particularly the rate of decomposition and the partitioning of C between the different soil CO_2 efflux sources are in the focus of soil science research, which can be studied in detail with the help of natural ^{13}C abundance method. Thus, analysing the isotopic composition of CO_2 exchange between the soil and the atmosphere not only helps to gain more information about the impact and role of SOM and its various forms but also to predict ecosystem responses to global changes.

Keywords: stable C isotopes, ^{13}C natural labelling, soil organic carbon turnover, isotope fractionation

Introduction

The application of stable isotope analysis has proved to be an extremely useful tool for tracking various changes in the Earth's systems. Since the discovery of isotopes in the 1910s, stable isotope geochemistry has provided essential information for geosciences, first for chemistry and geochemistry and later for biochemistry and ecology (Dawson, T.E. and Siegwolf, R.T.W. 2007). With the help of stable isotopes, paleo-environmental reconstruction became an achievable tool (Epstein, S. *et al.* 1953), as did the study of the atmosphere and the hydrological cycle via the isotopic signature of precipitation (Dansgaard, W. 1964). Stable isotopes also help the more precise identification of extinction events (Pálfy, J. *et al.* 2001). These are just a few examples of the possible application of stable isotopes in geochemical questions. Today, stable isotope analyses cover almost the entire spectrum of geoscience research and in some areas their application is mandatory (Deméný, A. 2004).

The carbon isotope composition of organic and inorganic compounds alters in the course of exchange processes in the vegetation-soil-atmosphere cycle, leaving an isotopic imprint on plant, soil and atmospheric carbon pools and fluxes (Werner, C. *et al.* 2012). These isotopic imprints allow, for example, the tracking of newly assimilated C incor-

[1] Geographical Institute, Research Centre for Astronomy and Earth Sciences, Hungarian Academy of Sciences, H-1112 Budapest, Budaörsi út 45. E-mail: zachary.dora@csfk.mta.hu
[2] Institute for Nuclear Research, Hungarian Academy of Sciences. H-4026 Debrecen, Bem tér 18/c.

porated in plants, then migrating to the soil, stored within the soil ecosystem or lost to the atmosphere (Brüggemann, N. *et al*. 2011). Paleo-environmental studies (Schwartz, D. *et al.* 1986; Cerling, T.E. *et al.* 1989; Fox, D.L. and Koch, P.L. 2004; Barta, G. *et al.* 2018) use stable carbon isotopes in soils or paleosols to track past changes in vegetation and climate. Stable carbon isotope methods related to landscape evolution, land use change and erosion studies (Paul, S. *et al.* 2008a; Häring, V. *et al.* 2013; Alewell, C. *et al.* 2016; Brandt, C. *et al.* 2016, 2018) make it possible to trace the origin and stability of carbon in different systems during diverse processes.

Today, stable isotope information permits scientists to address issues that seemed intractable using other methods. The stable isotope data generated with these methods have provided insights into a wide range of complex processes on temporal and regional scales from seconds to millennia and from cells to net ecosystem flux partitioning (Dawson, T.E. *et al.* 2002; Dawson, T.E. and Siegwolf, R.T.W. 2007).

This review provides the theoretical background of stable isotope research, using examples of major processes resulting in carbon isotope fractionation to illustrate various types of isotope fractionation and highlighting the stable carbon isotope variation in the Earth's main reservoirs, focusing in particular on soil ecosystems. It details the applicability of stable carbon isotope research in soil sciences, with special attention to the ^{13}C natural labelling approach. The natural abundance of stable carbon isotopes has been widely used to probe the turnover of SOM and to differentiate the diverse sources of CO_2 efflux from the soil. These results provide a clearer picture of the fate of organic carbon in the vegetation-soil-atmosphere cycle.

Isotope nomenclature and fractionation

Determining the absolute abundance of isotopes is difficult, because absolute variations in isotopic abundance based on physical and biological factors are small (to the order of a few percent) (Ehleringer, J.R. and Rundel, P.W. 1989), so relative isotope abundance is conventionally calculated as follows:

$$R = \frac{rare\ isotope}{abundant\ isotope}, \quad (1)$$

where R is the ratio of the *rare isotope* to the *abundant isotope*. The ratio R of a sample is generally compared to that of a known standard material, which provides high precision and repeatability over the long-term. Because of the small variations present in nature between the isotopic compositions of the sample and the standard material, the ratios are expressed using the conventional δ notation, introduced by Craig, H. (1953), in parts per thousand:

$$\delta\ (‰) = \frac{R_{sample}}{R_{standard}} - 1\ (\text{x}\ 10^3) \quad (2)$$

The unit of δ is "‰" or "permil" (also per mill).

The worldwide standards for the six conventional elements are V-SMOW (Vienna-Standard Mean Ocean Water) for H, V-PDB (Vienna-PDB, a replacement standard for the original calcium carbonate found in *Belemnitella americana* in the Cretaceous PeeDee formation in South Carolina, USA) for C, AIR N_2 for N, V-SMOW for O, V-CDT (Troilite from the Canyon Diablo iron meteorite) for S and NBS-28 (quartz sand) for Si (Hoefs, J. 2009).

The basis for isotope geochemistry is the fractionation of isotopes, i.e. 'the partitioning of isotopes between two phases of the same substance with different isotope ratios' (Hoefs, J. 2009), which results in different isotopes of the same element having different distribution patterns in the environment. The fractionation factor (α) is the difference in the ratio of the product isotope ratio (R_p) to the reactant isotope ratio (R_R):

$$\alpha = \frac{R_P}{R_R} \quad (3)$$

In general, isotope effects are small, $\alpha \approx 1$, so it has become common practice in recent years to replace the fractionation factor α by the deviation of α from 1, referred to as the *ε-value*:

$$\varepsilon = \alpha - 1 \ (\times 10^3) \qquad (4)$$

The *ε-value* represents the enrichment ($\varepsilon > 0$) or depletion ($\varepsilon < 0$) of the rare isotope in the product compared to the reactant isotope and approximates the fractionation in parts per thousand, making it similar to the *δ value* (Mook, W.G. 2001; Hoefs, J. 2009).

Isotope fractionation is often referred to as 'discrimination' in biological systems, meaning that specific enzymes discriminate against the heavier and favour the lighter isotope (Dawson, T.E. *et al.* 2002).

Isotope fractionation is caused by mass-dependent and mass-independent mechanisms, of which the latter is less frequent. Isotope exchange reactions (or equilibrium isotope distribution) and kinetic processes are the main mass-dependent processes.

Equilibrium isotope fractionation

The distribution of isotopes is controlled by the lowest energy state of the system (Ohkouchi, N. *et al.* 2015; Trumbore, S.E. *et al.* 2016). The energy state of a molecule is based differences in translation, rotation and vibration energy, among which differences in vibrational energy are predominant. Therefore, this is the source of isotope partitioning (Hoefs, J. 2009). The vibrational energy of a molecule depends inversely on the masses of the atoms in the molecule (Bigeleisen, J. 1965). As a consequence, isotopes partition differently for various types of chemical bonds and for the phases of the same molecule (e.g. for H_2O as vapour, liquid or ice). The heavier isotope prefers molecules with stronger bonds and phases with less entropy (e.g. a solid versus a liquid versus a gas) (Trumbore, S.E. *et al.* 2016).

Equilibrium isotope fractionation occurs in nature especially between the phases of the $CO_2 - H_2O - H_2CO_3 - CaCO_3$ system. One typical example is the isotope equilibrium between gaseous CO_2 and dissolved bicarbonate (HCO_3^-):

$$^{13}CO_{2(gas)} + H^{12}CO_3^- \leftrightarrow {}^{12}CO_{2(gas)} + H^{13}CO_3^- \qquad (5)$$

In this fractionation the ^{13}R (see above) is 0.0111421 for CO_2 gas (g) and 0.0112372 for bicarbonate (b) at 20 °C (Mook, W.G. 2001), so the fractionation factors are $^{13}\alpha_{g/b} = 0.9915$ and $^{13}\varepsilon_{g/b} = -8.46‰$. *Figure 1.* illustrates the different *ε-values* for different phases of the $CO_2 - H_2O - H_2CO_3 - CaCO_3$ system. This figure also shows the temperature dependence of isotope fractionation. In general, isotope fractionation is higher at a lower temperature, while it becomes zero at a very high temperature, based on the different vibrational frequencies of the molecules (Hoefs, J. 2009).

Another example of equilibrium fractionation is the precipitation of calcium carbonate from water. In this case the heavier C isotope will partition into the calcium carbonate, which has fewer degrees of freedom because it is solid. The $\delta^{13}C$ of C in calcium carbonate will be enriched to a greater extent (~10‰)

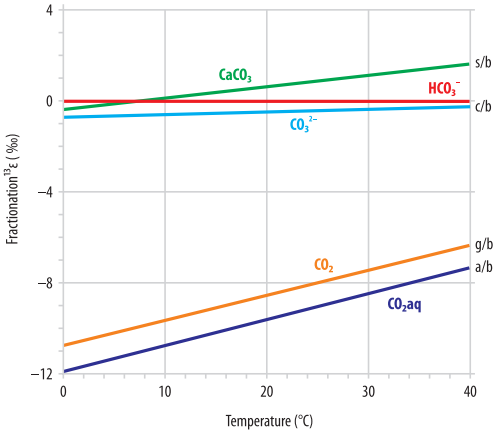

Fig. 1. Temperature-dependent equilibrium isotope fractionation for the different phases of the $CO_2 - H_2O - H_2CO_3 - CaCO_3$ system. – a = dissolved CO_2; b = dissolved HCO_3^-; c = dissolved carbonate ions; g = gaseous CO_2; s = solid carbonate. The different phases are shown with respect to dissolved HCO_3^-. *Source*: redrawn from Mook, W.G. 2001.

than that of atmospheric CO_2 in equilibrium with water from which the calcium carbonate is precipitated (Mook, W.G. 2001; Trumbore, S.E. *et al.* 2016).

Kinetic isotope fractionation

In comparison with equilibrium fractionation, kinetic fractionation occurs in non-equilibrium conditions when a reaction is irreversible, such as evaporation, diffusion, dissociation or biologically mediated reactions (Bigeleisen, J. and Wolfsberg, M. 1958; Hoefs, J. 2009; Ohkouchi, N. *et al*. 2015). Kinetic processes depend primarily on differences in the reaction rates of isotopic molecules: the lighter isotope will react and diffuse faster than the heavier isotope at a given temperature (Hoefs, J. 2009). As a consequence, the preferential enrichment of the lighter isotope is observed in the reaction products compared to the heavier isotope (Mook, W.G. 2001; Michener, R.H. and Lajtha, K. 2007; Hoefs, J. 2009; Ohkouchi, N. *et al*. 2015; Trumbore, S.E. *et al.* 2016).

One prominent example of stable C isotope fractionation is the process of photosynthesis:

$$6CO_2 + 6H_2O + light \rightarrow C_6H_{12}O_6 + 6O_2, \qquad (6)$$

where the ^{13}R of the reactant (atmospheric CO_2) = 0.9926, and the ^{13}R of the product (plant material) = 0.9724 (Trumbore, S.E. *et al.* 2016), giving fractionation factors of $^{13}\varepsilon = 0.9796$ and $^{13}\alpha = -20.4‰$.

Another kinetic process is the mineralization (bacterial decomposition) of soil organic matter to methane, resulting in an *ε-value* of about −55‰. Although natural processes are not purely kinetic or irreversible, they are often referred to as non-equilibrium fractionations (Mook, W.G. 2001).

Mass-independent fractionation

Some fractionation processes do not exhibit the mass-dependent effects described above. Mass-independent fractionation was observed in meteorites by Clayton, R.N. *et al.* (1973) with the use of oxygen isotope diagrams and was interpreted by Thiemens, M.H. (1999). In this kind of fractionation, Allègre, C.J. (2008) reported that isotope differences do not depend on the mass difference but on the symmetry of the molecule. Mauersberger, K. *et al.* (1999), however, demonstrated experimentally that it is not the symmetry of the molecule which is responsible for fractionation but the difference in its geometry. New research indicates that mass-independent isotope fractionations are more abundant than originally thought and serve as a novel form of the isotopic fingerprint (Hoefs, J. 2009).

Stable carbon isotope variation in the Earth's reservoirs

Of the three naturally occurring C isotopes, ^{12}C and ^{13}C are stable, representing 98.89% and 1.11% of the C atoms on Earth, respectively (Meija, J. *et al.* 2016). Both stable isotopes were originally created by nucleosynthesis in stars and their abundance has remained constant since their synthesis (Trumbore, S.E. *et al.* 2016). However, the relative abundance of stable C isotopes may vary in the Earth's various carbon reservoirs (atmosphere, biosphere, hydrosphere, lithosphere), resulting in naturally occurring variations greater than 120‰, from heavy marine carbonates ($\delta^{13}C$ values +20‰) to light methane ($\delta^{13}C$ values –110‰, *Figure 2*). The systematic differences in the $\delta^{13}C$ values of various carbon reservoirs have been known since the work of Nier, A.O. and Gulbransen, E.A. (1939).

Stable carbon isotope studies in soil science

Norman, A.G. and Werkman, C.H. (1943) conducted the first soil tracer study on ^{15}N-labelled soybean residues, examining their decomposition in the soil. Since then many types of research have used tracers to track the fate of SOM constituents and dynamics in soils. Stable

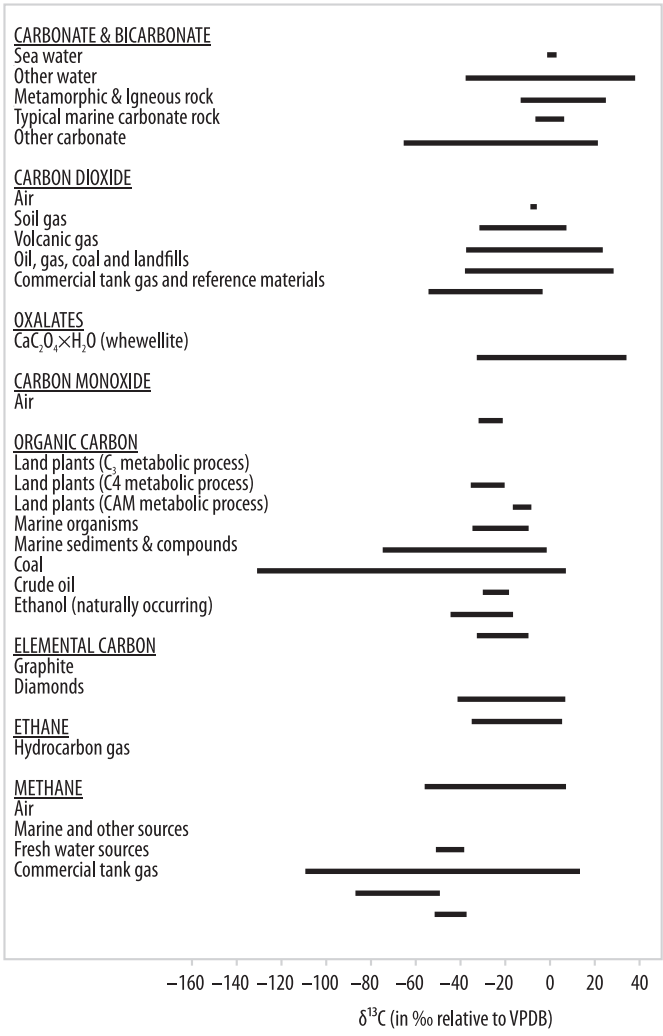

Fig. 2. $\delta^{13}C$ variations in selected carbon-bearing materials. *Source*: redrawn from Meija, J. *et al.* 2016.

carbon isotope measurements in soil science studies have become more and more significant in the past decades, as soil plays an important role in the global carbon cycle.

The significance of stable carbon isotope research in soil science was summarized by Brüggemann, N. *et al.* (2011), who provided a comprehensive overview of the complex network of carbon transformation and transport processes in the plant-soil-atmosphere continuum and demonstrated that research using C isotopes makes it possible to track the fate of C molecules and to integrate information on physical, chemical and biological processes in ecosystems across space and time. Kuzyakov, Y. (2011) stated that isotopic tracers are the most frequently applied and most powerful tracers because of the nearly identical chemical and biochemical properties of isotopes of a single element.

Isotope labelling in soils is based on the fact that biological, chemical and physical

fractionation processes in nature are uniquely $\delta^{13}C$ labelled, and that this labelling is inherited in the soil. This labelling happens naturally. Another technique for understanding C dynamics in soils is to artificially alter the C isotope content of assimilated C using enriched stable (^{13}C) or radioactive (^{14}C) C compounds (CO_2, whole plant residues or plant monomers and polymers) in short pulses (pulse labelling) or over long periods (continuous labelling) (Kuzyakov, Y. 2006).

Stable C isotope fractionation processes in the atmosphere-plant-soil system

The atmospheric CO_2 photosynthesis of plants and the different mechanisms involved were reported by Bender, M.M. (1971), who was the first to describe differences in the $\delta^{13}C$ values of various plant species. Reviews published from the 1980s onwards (e.g. O'Leary, M.H. 1981; Farquhar, G.D. *et al.* 1989; Hayes, J.M. 2001) provided the biochemical background of carbon isotope fractionation during photosynthesis. It was concluded that there are three different mechanisms of photosynthetic CO_2 fixation: the C_3 (Calvin-Benson) pathway, the C_4 (Hatch-Slack) pathway and the crassulacean acid metabolism (CAM). Plant photosynthesis strongly discriminates against the heavier carbon isotope, so the uptake of this isotope by C_3 and C_4 plants averages 19‰ and 4‰ less, respectively, than the atmospheric ambient $\delta^{13}C$ *(Figure 3)* (Boutton, T.W. 1996; Hoefs, J. 2009), which is −8‰ compared to the V-PDB standard (see *Figure 2* and *3*). The CAM pathway is a modification of photosynthetic carbon fixation resulting in $\delta^{13}C$ values ranging from –10 to –28‰ (Boutton, T.W. 1996).

After CO_2 photosynthetic fixation by plants, further fractionation processes take place, resulting in different $\delta^{13}C$ values for different compounds in plants (Park, R. and Epstein, S. 1960). Lignin, lipids and cellulose are depleted, while sugars, amino acids and hemicelluloses are enriched in ^{13}C relative to the bulk plant material (Boutton, T.W. 1996). Therefore, within a single plant $\delta^{13}C$ differences between substances may be as much as 9‰ for C_3 plants and 10.3‰ for C_4 plants (Hobbie, E.A. and Werner, R.A. 2004). Kinetic isotope effects seem to be the cause of these ^{13}C differences (Hoefs, J. 2009).

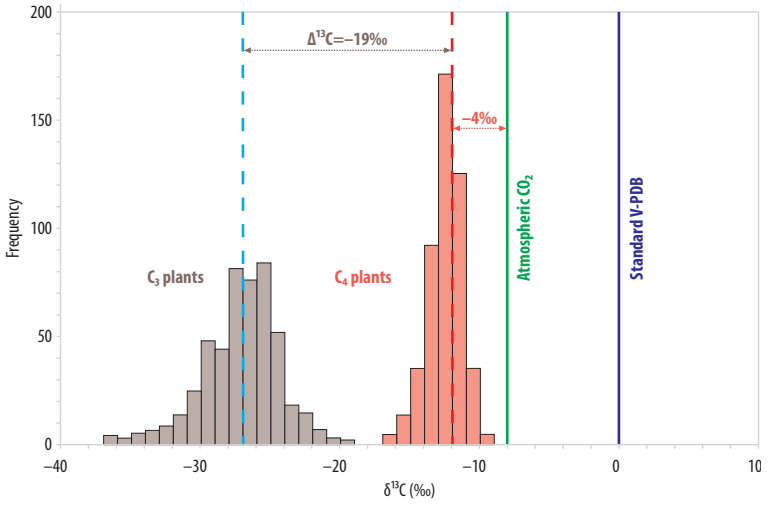

Fig. 3. Isotopic composition of C_3 and C_4 plants compared to atmospheric CO_2 and the C isotope ratio measurement standard. *Source*: redrawn from Ehleringer, J.R. and Cerling, T.E. 2002.

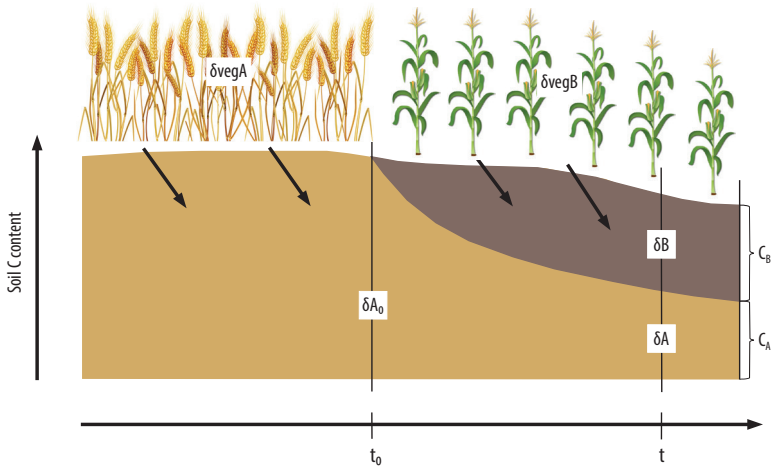

Fig. 4. The basis of C_3–C_4 vegetation change for natural abundance ^{13}C labelling. The figure represents the replacement of SOM derived from previous vegetation A by the new vegetation B. *Source:* redrawn from Balesdent, J. and Mariotti, A. 1996.

In addition, microbes in the soil also discriminate isotopes. The term 'preferential substrate utilization' or 'preferential decomposition' refers to the phenomenon whereby microorganisms select certain individual substances in plant residues and decompose them to CO_2 (Werth, M. and Kuzyakov, Y. 2010). Microbes, especially the bacteria prefer easily decomposable substances (e.g. glucose, sucrose) enriched in ^{13}C rather than lignin and lipids. This preferential substrate utilization is more significant than the ^{13}C-depletion effect of the metabolism (CO_2 from microbial respiration is ^{13}C-depleted compared to the substrate from which it is derived) (Šantrůčková, H. *et al.* 2000). As a consequence, the CO_2 emitted during decomposition is enriched in ^{13}C, while ^{13}C-depleted SOM remains in the soil, as the preferential utilization of the ^{13}C-enriched SOM fractions means that ^{13}C is lost more rapidly than ^{12}C (Ågren, G.I. 1996).

Application of natural C isotope fractionation

The differences in $\delta^{13}C$ values between C_3 and C_4 plants have great importance for soil science since the $\delta^{13}C$ of SOM in the steady-state system is nearly identical to that of the source vegetation from which the organic matter was derived (Boutton, T.W. 1996). This is the basis for numerous stable carbon isotopic applications in soil science.

The natural labelling or $\delta^{13}C$ natural abundance method is based on 1) the above-mentioned physiological difference in the photosynthetic fixation of CO_2 in C_3 and C_4 plants and 2) the assumption that the $\delta^{13}C$ natural abundance signature of SOM is identical to the $\delta^{13}C$ natural abundance signature of the plants from which it is derived, because the isotopic difference between C_3 and C_4 plants is much larger than the isotopic changes occurring during SOM decay (Balesdent, J. and Mariotti, A. 1996). Thus, growing C_4 plants on a C_3 soil or vice versa can be considered as *in situ* labelling. With this method the rate of loss of the C derived from the original vegetation and the incorporation of C derived from the new vegetation can be estimated (Balesdent, J. *et al.* 1987). As a consequence, the natural labelling approach makes it possible 1) to calculate the turnover rate of C derived from the original vegetation (Six, J. and Jastrow, J. 2002) and 2) to separate the different sources of soil CO_2 efflux (Kuzyakov, Y. 2006).

In natural labelling experiments, in contrast to artificial labelling, the isotope differences are smaller, but they have the advantage that no artificially enriched compounds are required. For example, as described by Gunina, A. and Kuzyakov, Y. (2014), the $\delta^{13}C$ natural abundance approach is able to estimate C flows under steady-state conditions without applying artificial tracers. In addition, the distribution of ^{13}C between the pools is more uniform than in artificial pulse labelling methods (Kuzyakov, Y. 2005). One of the strengths of the method is the easy application under field conditions, because there is no need for artificial labelling equipment or isolation from the atmosphere (Kuzyakov, Y. 2006). Therefore, this technique is one of the best for the study of field soil dynamics (Paul, E.A. 2016).

Nevertheless, the method has some shortcomings (Kuzyakov, Y. 2006): (i) C_3 plant/C_4 soil pairs or vice versa are rare under field conditions; (ii) the maximum $\delta^{13}C$ variation of CO_2 between C_3 and C_4 plants is only about 14‰; (iii) there is a ^{13}C discrimination by plants caused by temperature, water availability, air humidity, N supply, light intensity and plant properties (root length, plant sex).

Figure 4 illustrates the basis of the C_3–C_4 vegetation change method representing the two photosynthetic pathways A and B (Balesdent, J. and Mariotti, A. 1996). At the time of the vegetation change (t_0) SOM has an isotopic composition δ_{A0} close to that of the original vegetation.

As this SOM from vegetation A progressively decays, it is partially replaced by SOM derived from the new vegetation B. At a given time *t*, the total SOM content can be expressed as $C = C_A + C_B$ and the isotope composition δ_{AB} of SOM under mixed vegetation is the following:

$$\delta_{AB}\,(C_A + C_B) = \delta_{AB}\,(C) = \delta_A C_A + \delta_B C_B\,, \quad (7)$$

where C_A and C_B stand for the amount of SOM from the old (*A*) and new (*B*) vegetation, respectively, and δ_A and δ_B are the $\delta^{13}C$ values of SOM derived from vegetation A and B, respectively. As $C_A = C - C_B$, Eq. (7) can be rewritten as follows (Amelung, W. *et al.* 2008):

$$\delta_{AB} = \frac{\delta_B C_B}{C} + \frac{\delta_A (C - C_B)}{C} = \frac{\delta_B C_B}{C} + \delta_A \left(1 - \frac{C_B}{C}\right) \quad (8)$$

Hence, the contribution of plant B to the total C content can be calculated as follows (Balesdent, J. and Mariotti, A. 1996; Amelung, W. *et al.* 2008):

$$F = \frac{C_B}{C} = (\delta_{AB} - \delta_A) / (\delta_B - \delta_A) \quad (9)$$

expressed as the fraction of new carbon in the soil (*F*).

Because δ_A and δ_B cannot be measured directly in the mixed cropping system, they must be estimated. The natural labelling method assumes that δ_B is equivalent to the isotopic composition of the new vegetation ($\delta_{VEG\,B}$, see *Figure 4*), and δ_A to the initial $\delta^{13}C$ of the soil or of the control soil remaining under the initial vegetation ($\delta_{REF\,A}$). Hence, the new portions of vegetation B are estimated as follows (Balesdent, J. and Mariotti, A. 1996; Amelung, W. *et al.* 2008):

$$F = (\delta_{AB} - \delta_{REF\,A}) / (\delta_{VEG\,B} - \delta_{REF\,A}) \quad (10)$$

Many studies (Balesdent, J. and Balabane, M. 1992; Six, J. *et al*. 1999; Dignac, M.F. *et al.* 2005; Paul, E.A. *et al*. 2008b; Pausch, J. and Kuzyakov, Y. 2012; Schiedung, H. *et al*. 2017; Poeplau, C. *et al*. 2018) have applied the ^{13}C natural abundance approach to calculate the proportion of C derived from the new vegetation/fresh organic input. Based on Eq. (10), this technique allows also the percentage of C derived from different treatments and amendments to be calculated. For example, Lynch, D.H. *et al*. (2006) estimated the percentage of C derived from different C_4 compost treatments and the retention of compost C in a temperate grassland (C_3) soil in Nova Scotia. Measurements took place one and two years after the application of the compost treatments (corn silage, dairy manure and sewage sludge) and showed that

the fraction of SOM derived from compost was around 33% for most of the treatments. The results indicated that the fraction of compost retained in the soil was the highest for corn silage compost one and two years after the treatment (~ 95% and 90%, respectively).

Another possible application of the ^{13}C natural abundance method is connected with land use change studies (e.g. Yamashita, T. *et al*. 2006; Jakab, G. *et al*. 2018a; Zhang, Q. *et al*. 2018). Agricultural land use disturbs the natural SOM system, e.g. by affecting the aggregate size and stability of SOM (Bilandžija, D. *et al*. 2017; Jakab, G. *et al*. 2018b). With the help of ^{13}C natural abundance these effects can be examined in more detail, as stable carbon isotopes widen the scope of land use research. For instance, the effect of land use changes on the aggregate systems in the soil or how different land use types influence the fraction of C derived from the new vegetation can be examined with the help of ^{13}C natural abundance approach (John, B. *et al*. 2005; Yamashita, T. *et al*. 2006; Paul, S. *et al*. 2008a,b; Liu, Y. *et al*. 2018).

In addition, ^{13}C natural abundance has been successfully applied to trace sediment and SOM transfer during erosion (Papanicolaou, A.N. *et al*. 2003; Alewell, C. *et al*. 2008; Schaub, M. and Alewell, C. 2009; Zollinger, B. *et al*. 2014). The source of eroded soil sediments or suspended organic matter and the rate of soil erosion and redistribution can be monitored by the $\delta^{13}C$ signature of soils. Turnbull, L. *et al*. (2008) used the $\delta^{13}C$ signals of eroded material of soils over a C_4 grass to C_3 shrub transition. They concluded that variations in $\delta^{13}C$ values of SOM in bulk eroded sediment can be used to trace changes in erosion dynamics over events of different magnitudes and over different vegetation types. Jacinthe, P.A. *et al*. (2009) determined the amount and source of eroded soil organic carbon retained in C_3 grass filters receiving runoff from areas supporting C_4 vegetation. Novara, A. *et al*. (2015) measured the $\delta^{13}C$ values of different soil profiles sampled along a Sicilian vineyard slope and quantified the rates of erosion.

Estimation of the turnover rate of C pools

The carbon turnover rate is the rate of C cycling from one pool to another. If the system is in the steady-state condition (i.e. input into the pool is equal to the output), the value of the turnover rate is the ratio of the input amount per time unit to the total pool amount. In this case, the mean residence time (i.e. the mean period of residence of C in the given pool) is the inverse of the turnover rate (Kuzyakov, Y. 2006).

Based on the simplest assumption, SOM consists of a homogeneous, single C pool, which decomposes exponentially following first-order kinetics (Stanford, G. and Smith, S.J. 1972). For the amounts of SOM from the old vegetation:

$$C_A = (C_A + C_B) \exp(-kt), \qquad (11)$$

where C_A and C_B stand for the amount of SOM from the old (A) and new (B) vegetation, k is the decay rate constant and t is the time since vegetation change. The mean residence time (*MRT*) can be calculated as the inverse of the decay rate constant as follows (Amelung, W. *et al*. 2008):

$$MRT = \frac{1}{k} = -t/\ln(1 - F) \qquad (12)$$

SOM pools dominated by turnover times ranging from a year to several hundreds of years have been calculated with the help of the natural labelling approach (Balesdent, J. and Mariotti, A. 1996).

Carbon turnover time is not just an important indicator of SOM dynamics, but is a key parameter in coupled climate-carbon cycle models (e.g. Earth System Models). Hence, there is an urgent need to accurately estimate the turnover times of SOM to predict the future sizes of the terrestrial C sinks and sources and to obtain a better understanding of climate-carbon feedback (Carvalhais, N. *et al*. 2014; He, Y. *et al*. 2016; Wang, J. *et al*. 2018).

Balesdent, J. *et al*. (1987) were the first to use the natural ^{13}C abundance method on two French sites which originally had C_3 type veg-

etation. They cultivated maize (C_4 type vegetation) to achieve a C_3/C_4 vegetation change. An organic carbon turnover rate of 22% was calculated from the $\delta^{13}C$ values of soils sampled at one experimental site after 13 years of maize cultivation, with different annual rates. This suggested that the decay of SOM cannot be described using a single carbon pool model. A turnover time of 36 years was calculated for this site assuming an exponential decay. At another experimental site, where continuous maize cultivation for 23 years was applied after pine forest clearing, two treatments were used: in the first, leaves and stalks were incorporated back into the soil, while in the second, leaves and stalks were removed for the last 17 years. The percentage of organic carbon derived from maize was calculated for different particle size fractions in the topsoil (0–30 cm) and subsoil (30–40 cm) horizons. The turnover of the coarse sand fraction (200–2,000 mm) was found to be the most rapid, while the fine clay fraction (<0.2 mm) contained most of the SOM.

Since then, a number of studies have used the ^{13}C natural abundance method for SOM turnover rate calculations for different purposes, but the work of Balesdent, J. *et al.* (1987) forecast the major questions of SOM research which have since been studied with this method. These are the 1) estimation of the turnover time of different physically and/or chemically separated SOM fractions representing distinct SOM fractions connected to different soil textures or minerals (Martin, A. *et al*. 1990; Bonde, T.A. *et al*. 1992; Balesdent, J. *et al*. 1998; Shang, C. and Tiessen, H. 2000; Liao, J.D. *et al*. 2006; Dalal, R.C. *et al*. 2013); 2) estimation of the turnover time of different SOM pools (Bernoux, M. *et al.* 1998; Derrien, D. and Amelung, W. 2011); 3) estimation of the turnover time of SOM derived from different treatments or land uses (Six, J. and Jastrow, J. 2002; Zach, A. *et al*. 2006; Novara, A. *et al*. 2013); 4) comparison of the turnover time of SOM at different soil depths (Bernoux, M. *et al*. 1998; Flessa, H. *et al*. 2017).

A combination of these topics is embedded in many other studies. For example, Collins, H.P. *et al.* (1999) investigated the soil C dynamics in the Corn Belt region of the central USA. They calculated the per cent of C derived from corn after conversion to a monoculture of C_4 corn and the MRTs of the C_3 soils. The proportion of corn-derived C decreased with soil depth and was minimal in the 50–100 cm depth increments of fine-textured soils. The mean residence time of non-corn C (C_3) ranged from 36 to 108 years at the surface and up to 769 years at the subsoil depth. It was shown that clay minerals effectively protected the organic matter in the case of older C_3-derived C (longer MRTs), while no such protection was observed for the younger C_4-derived C (shorter MRTs).

John, B. *et al*. (2005) estimated the turnover times of different density fractions of SOM (free particulate organic matter with a density <1.6 g cm^{-3}, light occluded particulate organic matter with a density of <1.6 g cm^{-3}, dense occluded particulate organic matter with a density of 1.6–2.0 g cm^{-3} and mineral-associated SOM with a density >2 g cm^{-3}) and of SOM from different depths. They calculated turnover times of 54, 144 and 223 years for the 0–30 cm, 30–45 cm and 45–60 cm horizons, respectively. The mean turnover times for the density fractions were found to be the following: 22 years for the free particulate organic matter, 49 years for the dense occluded particulate organic matter, 63 years for mineral-associated SOM and 83 years for light occluded particulate organic matter.

Lisboa, C.C. *et al*. (2009) calculated the turnover time of different SOM fractions (>250 mm, 53–250 mm, 2–53 mm, <2 mm) applying a two-pool (active and slow decomposition rate) exponential model for a forest-to-pasture chrono-sequence in the Brazilian Amazon. Except for the >250 mm fraction no difference was detected between the fractions in the active pool phase, whereas in the slow pool phase the fractions were separated according to their turnover rates: the clay-associated SOM (fraction <2 mm) had the greatest turnover rate (>2,500 years), the microaggregate and silt-associated SOM had medium turnover rates (498 and 210 years,

respectively) and the particulate organic matter (>250 mm fraction) had the smallest turnover rate (~ 1 year).

Panettieri, M. *et al*. (2017) studied the different turnover times of fractionated water-stable aggregates (larger macro-aggregates with 2.0–7.1 mm, macro-aggregates with 0.200–2.00 mm, microaggregates with 0.050–0.200 mm and silt + clay fraction with <0.050 mm) of permanent cropland and temporary grassland plots after nine and three years of maize cultivation, respectively. The calculated turnover times for the two land uses were similar for the micro- and macro-aggregates but different for the silt + clay fraction. Namely, the MRT of the silt + clay fraction of grassland soil was twice as of the cropland soil confirming that this smallest fraction is affected to the greatest extent by land use practices, and particularly tillage. It could be explained by the increased degradation of SOM due to higher aeration caused by tillage.

Soil CO_2 efflux source determination

Besides the estimation of SOM turnover time, the ^{13}C natural abundance method is well applicable to partition the CO_2 fluxes from the soil (Cheng, W. 1996). The evaluation of the contribution made by different C sources to soil CO_2 efflux is also a key parameter in determining whether the soil is a net source or sink of atmospheric CO_2 (Kuzyakov, Y. and Larionova, A.A. 2005).

According to Kuzyakov, Y. (2006) there are five main sources of soil CO_2 efflux *(Figure 5)*: 1) microbial decomposition of SOM (termed basal respiration), 2) microbial decomposition of SOM affected by recent input of rhizodeposits and/or fresh undecomposed plant residues (termed priming effect), 3) microbial decomposition of partly decomposed dead plant remains, 4) microbial decomposition of rhizo-deposits of living roots (termed rhizomicrobial respiration) and 5) root respiration (respiration of assimilates by roots of autotrophic plants). These CO_2 effluxes represent different C pools with different turnover rates and MRTs (see *Figure 5*).

The pedogenic or anthropogenic acidification of soils containing $CaCO_3$ is also a source of CO_2 efflux in the soil, but its contribution is only significant on the geological time scale and not on the sub-annual to decadal time scales used in soil research studies (Kuzyakov, Y. 2006).

With the help of the ^{13}C natural labelling approach, it is possible to separate the sources of soil respiration. Growing C_4 plants on a C_3 soil or vice versa and tracing the $\delta^{13}C$ value of CO_2 efflux from the soil allows the separation of SOM-derived from plant-derived CO_2 (see *Figure 5*). If additional data on the $\delta^{13}C$ values of microbial biomass and roots is available, root and rhizomicrobial respiration can also be partitionated, as can the separation of SOM-derived basal respiration from the microbial decomposition of plant residues.

Natural C_3/C_4 vegetation differences are also used to partition the autotrophic (root respiration) and heterotrophic (other 5 respiration sources in *Figure 5*) soil respiration in many studies (e.g. Rochette, P. and Flanagan, L.B. 1997; Giardina, C.P. *et al.* 2004; Millard, P. *et al.* 2008).

Millard, P. *et al.* (2010) were the first to quantify the proportion of SOM-derived CO_2 in a forest soil using ^{13}C natural abundance discrimination, with carbon input derived solely from C_3 photosynthesis. For this, measured $\delta^{13}C$ values of root respiration (−27.60 ± 0.51‰) and SOM-derived respiration (−25.10 ± 0.88‰) were used as the end points of a two-component mixing model using the small isotopic difference between them. The calculated mean percentage of SOM-derived CO_2 was 0.61 ± 0.28.

By adding C_4 plant residues to a C_3 soil or vice versa and measuring their contribution to the total CO_2 efflux it is also possible to separate the CO_2 originating from plant residues and that derived from the microbial decomposition of SOM. In addition, by comparing soil with added residues to control soil with no residue addition, the priming effect can be calculated using the ^{13}C natural labelling approach (Kuzyakov, Y. 2006). For

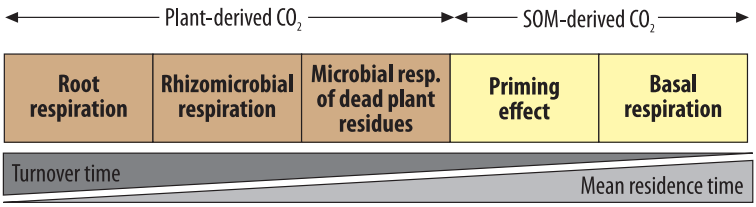

Fig. 5. Main sources of soil CO_2 efflux and C pools in order of turnover rates and residence times. *Source*: redrawn from Kuzyakov, Y. and Gavrichkova, O. 2010.

example, Kuzyakov, Y. and Cheng, W. (2001) applied the ^{13}C natural abundance method to partition the soil-derived and root-derived (root respiration plus rhizomicrobial respiration) CO_2 from C_4 prairie soil planted with C_3 wheat in a 7-day laboratory experiment. Photosynthesis was greatly reduced and, on average, 75% of total CO_2 efflux from the soil proved to be root-derived and 25% soil-derived. When the priming effect was compared for planted and non-planted soils a positive priming effect (42 mg C kg^{-1} h^{-1} and 33 kg C ha^{-1} d^{-1}) was recorded during the first 3 days, whereas without light, the priming effect decreased and was negative due to the reduction of exudation.

Werth, M. and Kuzyakov, Y. (2009) used the natural ^{13}C labelling approach to partition root respiration, rhizomicrobial respiration and basal respiration under field conditions in a loamy Haplic Luvisol in Stuttgart, Germany. They used the $\delta^{13}C$ values of SOM, roots, microbial biomass and total CO_2 efflux from the soil and applied isotopic mass balance equations to calculate the contributions of the three sources of CO_2 efflux. The $\delta^{13}C$ values from a bare-fallow plot were used to calculate the ^{13}C fractionation between SOM and CO_2 and between microbial biomass and CO_2 and the contribution of different CO_2 sources was estimated, taking into account the ^{13}C fractionation.

The calculations revealed significant changes between the results with and without ^{13}C fractionation. It was therefore suggested that the isotope fractionation processes of ^{13}C should be embedded in studies dealing with CO_2 efflux partitioning. Werth, M. and Kuzyakov, Y. (2010) reviewed the possible uncertainties connected with ^{13}C fractionation in the ^{13}C natural abundance method, with special attention to the partitioning of CO_2 efflux. It was concluded that even a small variation (±1.0‰) in the $\delta^{13}C$ value of the 'endmembers' of the mixing equations led to strong uncertainties. In addition, if significant isotope fractionation takes place, the uncertainties increase significantly. As possible solutions, they recommended various approaches to reduce uncertainties: 1) to increase the difference in $\delta^{13}C$ value between the two 'endmembers' (if necessary, using artificial labelling); 2) to estimate the fractionation of individual processes in the specific study, not using mean values estimated in other studies; 3) to analyse the $\delta^{13}C$ values of individual substance groups or substances (i.e. compound-specific isotope analysis).

Conclusions

The ^{13}C natural abundance approach occupies an important place among the isotope applications used in soil research, especially for the calculation of SOM turnover and the partitioning of CO_2 efflux sources.

The ^{13}C natural abundance approach combined with other methods is a useful tool to measure the effect of different human-induced changes on organic carbon storage, such as land use change, erosion and soil management. Along with the traditional methods of watershed monitoring, slope

measurements and rainfall simulation experiments or tracer applications (rare earth elements or radionuclides), stable carbon isotope measurements provide additional spatial information on soil erosion dynamics. In addition, the ^{13}C natural abundance approach in combination with photogrammetry or remote sensing could be useful to precisely monitor areas affected by different land use changes.

With the combination of ^{13}C natural abundance method and ^{14}C labelling, the contribution of carbon sources to the carbon pools can be distinguished in more detail and the priming effect connected to the processes can be calculated. The measurement of the natural ^{14}C abundance of SOM extends the timescales for C cycling to millennia supplementing the turnover times ranging from a year to several hundreds of years calculated by the ^{13}C natural labelling approach. The physical fractionation of soils combined with isotope labelling provides another possibility to estimate the turnover times of physically defined SOM pools.

Data obtained using the ^{13}C natural abundance technique provide important information on SOM dynamics, which has been in the focus of interest in recent years due to the significant role of soil in the global carbon cycle. The determination of the turnover time and size of the active and passive soil reservoirs is essential for the evaluation of whether they serve as potential sources or sinks for atmospheric CO_2. Therefore, techniques such as the ^{13}C natural abundance approach, not only lead to a better understanding of processes in the global carbon cycle but also provide fundamental information for climate change mitigation.

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