Soil Biology & Biochemistry 42 (2010) 1372–1384

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Review

^{13}C fractionation at the root–microorganisms–soil interface: A review and outlook for partitioning studies

Martin Werth [a,*], Yakov Kuzyakov [b]

[a] Institute of Systematic Botany and Ecology, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany
[b] Department of Agroecosystem Research, BayCEER, University of Bayreuth, D-95440 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 20 October 2009
Received in revised form
20 April 2010
Accepted 22 April 2010
Available online 8 May 2010

Keywords:
^{13}C natural abundance
^{13}C fractionation
C_3 and C_4 photosynthesis
FACE
Root respiration
Microbial utilization
Preferential substrate utilization
CO_2 partitioning
Rhizosphere
Dissolved organic matter
Carbon cycle

ABSTRACT

Natural variations of the $^{13}C/^{12}C$ ratio have been frequently used over the last three decades to trace C sources and fluxes between plants, microorganisms, and soil. Many of these studies have used the natural-^{13}C-labelling approach, i.e. natural $\delta^{13}C$ variation after C_3–C_4 vegetation changes. In this review, we focus on ^{13}C fractionation in main processes at the interface between roots, microorganisms, and soil: root respiration, microbial respiration, formation of dissolved organic carbon, as well as microbial uptake and utilization of soil organic matter (SOM). Based on literature data and our own studies, we estimated that, on average, the roots of C_3 and C_4 plants are ^{13}C enriched compared to shoots by $+1.2 \pm 0.6‰$ and $+0.3 \pm 0.4‰$, respectively. The CO_2 released by root respiration was ^{13}C depleted by about $-2.1 \pm 2.2‰$ for C_3 plants and $-1.3 \pm 2.4‰$ for C_4 plants compared to root tissue. However, only a very few studies investigated ^{13}C fractionation by root respiration. This urgently calls for further research. In soils developed under C_3 vegetation, the microbial biomass was ^{13}C enriched by $+1.2 \pm 2.6‰$ and microbial CO_2 was also ^{13}C enriched by $+0.7 \pm 2.8‰$ compared to SOM. This discrimination pattern suggests preferential utilization of ^{13}C-enriched substances by microorganisms, but a respiration of lighter compounds from this fraction. The $\delta^{13}C$ signature of the microbial pool is composed of metabolically active and dormant microorganisms; the respired CO_2, however, derives mainly from active organisms. This discrepancy and the preferential substrate utilization explain the $\delta^{13}C$ differences between microorganisms and CO_2 by an 'apparent' ^{13}C discrimination. Preferential consumption of easily decomposable substrates and less negative $\delta^{13}C$ values were common for substances with low C/N ratios. Preferential substrate utilization was more important for C_3 soils because, in C_4 soils, microbial respiration strictly followed kinetics, i.e. microorganisms incorporated heavier C ($\Delta = +1.1‰$) and respired lighter C ($\Delta = -1.1‰$) than SOM. Temperature and precipitation had no significant effect on the ^{13}C fractionation in these processes in C_3 soils. Increasing temperature and decreasing precipitation led, however, to increasing $\delta^{13}C$ of soil C pools.

Based on these ^{13}C fractionations we developed a number of consequences for C partitioning studies using ^{13}C natural abundance. In the framework of standard isotope mixing models, we calculated CO_2 partitioning using the natural-^{13}C-labelling approach at a vegetation change from C_3 to C_4 plants assuming a root-derived fraction between 0% and 100% to total soil CO_2. Disregarding any ^{13}C fractionation processes, the calculated results deviated by up to 10% from the assumed fractions. Accounting for ^{13}C fractionation in the standard deviations of the C_4 source and the mixing pool did not improve the exactness of the partitioning results; rather, it doubled the standard errors of the CO_2 pools. Including ^{13}C fractionations directly into the mass balance equations reproduced the assumed CO_2 partitioning exactly. At the end, we therefore give recommendations on how to consider ^{13}C fractionations in research on carbon flows between plants, microorganisms, and soil.

1. Introduction: the relevance of ^{13}C fractionation to root–microorganisms–soil interfaces

In the last three decades, a strong research interest has arisen to trace soil carbon (C) inputs and outputs. Besides artificial ^{14}C and ^{13}C labelling, the natural variation of the $^{13}C/^{12}C$ ratio in various terrestrial pools has often been used in C budgeting and C flow studies as

* Corresponding author. Tel.: +49 731 50 23310; fax: +49 731 50 23320.
E-mail address: martin.werth@uni-ulm.de (M. Werth).

0038-0717/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2010.04.009

well as in investigations into tracing C sources (see Meharg, 1994; Hanson et al., 2000; Ehleringer et al., 2000; Dawson et al., 2002; Hobbie and Werner, 2004; Kuzyakov and Larionova, 2005; Glaser, 2005; Subke et al., 2006; Bowling et al., 2008; Morgun et al., 2008; Amelung et al., 2008; Paterson et al., 2009 and others for further review). Those studies include C_3- to C_4-vegetation change or *vice versa*, decomposition studies with C_4-plant residues, incubation of naturally labelled compounds (i.e. sucrose or glucose originating from sugar cane or sugar beet), and maize-slurry applications, but also Free Air Carbon dioxide Enrichment (FACE), tree canopy labelling, continuous labelling by slightly enriched or depleted $^{13}CO_2$, etc. The most important processes involved in such studies are: root respiration, rhizodeposition, microbial uptake of plant compounds, microbial respiration, humification or stabilization of organic compounds, and some other indirectly relevant processes such as assimilate transport from shoots to roots.

Most chemical and biochemical processes favour the initial incorporation of the lighter isotope in the product, leaving the substrate enriched in the heavy isotope. This preference of one isotope in reactions is called 'isotopic effect'. It leads to differences between the isotopic composition of substrates and products (Högberg, 1997). The intensity of the isotopic effect is termed 'isotopic fractionation'. The magnitude of isotopic fractionation differs for various processes and depends on the specific reaction mechanism. In biological systems, isotope fractionation is also called 'discrimination' because specific enzymes discriminate against the heavier and favour the lighter isotope (Dawson et al., 2002). Thus, in studies based on ^{13}C natural abundance isotopic fractionation should be considered when calculating C partitioning ratios, C fluxes, and C budgeting.

Our review is focussed on ^{13}C fractionation by biotic processes during C flow from plant roots or plant residues to soil microorganisms and from soil organic matter to CO_2. Here, we do not review ^{13}C fractionation by photosynthesis and post-photosynthetic metabolic processes or by abiotic processes such as CO_2 diffusion through soil profiles, dissolution of CO_2 in soil water, carbonate precipitation, etc. These processes have been excellently reviewed by O'Leary (1981), Dawson et al. (2002), Hobbie and Werner (2004), and Morgun et al. (2008).

Our aim is to evaluate the most important fractionation processes at the interface between roots, microorganisms, and soil and to work out the consequences for studies based on small variations of the $^{13}C/^{12}C$ ratio (i.e. ^{13}C natural abundance), especially carbon partitioning studies. In this compilation we only review ^{13}C discrimination in processes under oxic conditions. ^{13}C discrimination under O_2 limitation contributing e.g. to the ^{13}C depletion in methane production was described by Conrad (2005).

2. Background

2.1. Definitions

Carbon has three naturally occurring isotopes (^{12}C, ^{13}C, and ^{14}C). ^{12}C and ^{13}C are stable C isotopes, whereas ^{14}C is radioactive. Their natural abundances are ca. 98.89% for ^{12}C, 1.11% for ^{13}C (Boutton, 1991a), and $<10^{-10}$% for ^{14}C (Goh, 1991) of the total carbon content in natural pools (air, plants, soil, etc.). Since the absolute variation in the natural stable carbon-isotope ratio R ($=^{13}C/^{12}C$) is small, sample C isotope ratios R_{sample} are expressed relative to the international PDB limestone standard as $\delta^{13}C$:

$$\delta^{13}C = \frac{R_{sample} - R_{PDB}}{R_{PDB}} 1000‰, \quad (1)$$

where R_{PDB} is the isotope ratio of the limestone fossil *Belemnitella americana* from the Cretaceous PeeDee Formation in South Carolina, which is set to $\delta^{13}C = 0‰$ as zero point reference. It has an absolute $^{13}C/^{12}C$ ratio of 0.0112372 (Craig, 1953).

Due to isotope effects during chemical reactions, isotopic fractionation occurs between a substrate ($R_{substrate}$) and a product ($R_{product}$) pool. This isotopic fractionation α is defined as:

$$\alpha = \frac{R_{substrate}}{R_{product}}. \quad (2)$$

For convenience, isotopic fractionations are more commonly reported as discrimination values Δ in ‰. α is related to Δ by:

$$\Delta = \alpha - 1. \quad (3)$$

These fractionations between a substrate and a product can be related to isotopic compositions through the following equation:

$$\Delta = \frac{\delta_{substrate} - \delta_{product}}{1 + \delta_{product}}, \quad (4)$$

where $\delta_{substrate}$ is the $\delta^{13}C$ value of the source and $\delta_{product}$ is the $\delta^{13}C$ value of the product (Lajtha and Michener, 1994). Since the denominator is mostly very close to 1, the simplified equation

$$\Delta \approx \delta_{substrate} - \delta_{product} \quad (5)$$

can also be used. Exact ^{13}C fractionations have to be determined in a single chemical reaction considering the $\delta^{13}C$ values of the substrates and products (Hobbie and Werner, 2004). In root respiration, for example, this consideration would include $\delta^{13}C$ values of the sugars involved in respiration for $\delta_{substrate}$ and of the respired CO_2 for $\delta_{product}$.

Most processes in the rhizosphere involve numerous individual reactions for which the determination of $\delta_{substrate}$ and $\delta_{product}$ of single compounds is hardly possible. Rhizosphere-related studies therefore tend to consider $\delta^{13}C$ values of bulk roots, soil organic matter (SOM), and/or microbial biomass instead of single compounds. It has to be noticed, however, that differences in $\delta^{13}C$ values between these bulk materials and the emitted CO_2 reflect various transformation processes. They involve their unique isotopic fractionations caused by biologically preferred utilization of ^{13}C-enriched (or -depleted) compounds and chemically faster or more slowly reacting isotopes (kinetic isotope effect). Hence, another measure often used is simply the isotopic difference between two pools, e.g. bulk roots and root respiration, defined as:

$$\Delta = \delta_{pool\ 1} - \delta_{pool\ 2} \quad (6)$$

According to Eq. (6), we will refer to positive Δ values (e.g. $\Delta = +3‰$) as ^{13}C enrichment of the considered pool (pool 1 for example is CO_2) compared to the source pool (pool 2 for example is roots), and to negative Δ values as ^{13}C depletion. This is in contrast to Eq. (5), where the source pool would be expressed by $\delta_{substrate}$ (i.e. by $\delta_{pool\ 1}$ − and not by $\delta_{pool\ 2}$ − as equivalent in Eq. (6)), but makes the fractionation processes in their description clearer.

2.2. Discrimination within the plants

Discriminations by the three photosynthesis pathways have been described in detail in many reviews (e.g. O'Leary, 1981; Farquhar et al., 1989) and books and are out of scope of this review. As the ^{13}C fractionation by C_3, C_4, and CAM photosynthesis provides the background for fractionation in further processes at the root–microorganisms–soil interface, we shortly repeat it here.

The $^{13}C/^{12}C$ ratio of organic carbon in terrestrial ecosystems is mainly influenced by the C isotope fractionation occurring during