



HAL
open science

Element cycling and organic matter turn-over (Chapter 3.2)

Christophe Robin

► **To cite this version:**

Christophe Robin. Element cycling and organic matter turn-over (Chapter 3.2). Jörg Luster; Roger Finlay. Handbook of Methods in rhizosphere research, pp.62-68, 2007, 3-905621-35-5. hal-04482576

HAL Id: hal-04482576

<https://hal.univ-lorraine.fr/hal-04482576v1>

Submitted on 28 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Editor: Robin, Christophe

UMR INRA-INPL(ENSAIA) "Agronomie et Environnement", BP 172,
F-54505 Vandoeuvre-les-Nancy Cedex, France

Christophe.Robin@ensaia.inpl-nancy.fr

3.2.1. Introduction

The turn-over of organic matter and element cycling is particularly difficult to assess in the field or in soil microcosms, especially in the rhizosphere, for which many challenges remain for research:

- the *in situ* quantification and localisation of the root released organic compounds;
- the identification, quantification of the different rhizosphere compartments (rhizosphere soil vs. bulk soil, rhizosphere soil vs. detritosphere, mycorrhizosphere, available compounds for microbial biomass, fungal vs bacterial biomass,...), and fluxes between compartments;
- the fate of those compounds in the different rhizosphere compartments (from plants to soil organic matter through biotransformation by microbial communities).

Nowadays different kind of methods are available. Among them, the following methodologies are covered in this chapter:

- for years, labelling techniques using stable and/or radioactive tracers are privileged and powerful tools to assess fluxes between compartments and / or the establishment of budgets
- additionally, isotope-based detection devices are the ideal tools for a global quantification of tracer in the plant-soil-microorganisms continuum
- promising techniques are under development for the quantification and localisation of root-released organic compounds; it includes the chemical characterization of organic compounds and the use of reporting biosensors.

Some important methods are not covered in this chapter because

- they represent refinements of the methods described here;
- they impose conditions where microorganisms are absent (sterile microcosms either with hydroponic systems or with sterilized substrates) and, therefore, are outside of the purpose of this chapter. Some of these methods are described in **chapter 3.1.**
- they are adapted to bulk soil, but either they lack of sensitivity in the case of rhizosphere soil or give rough estimates, without precise quantification of what really occurs in the rhizosphere (e.g.: indirect methods estimating the rhizomicrobial component of the rhizosphere respiration).

3.2.2. Labelling techniques

The methodologies involving isotopes (among them ^{13}C , ^{14}C , ^{11}C , ^{15}N , ^{32}P), are powerful research tools to assess the transfer and turn-over of elements in the plant, the soil and the microbial compartments. Overall, three tracer techniques are widely used to investigate the dynamics and budget of elements from atmosphere to the soil *via* the plant : 1) pulse-labelling, 2) continuous labelling, 3) natural abundance, each of them giving different information on the fate of C and N in the plant-soil system and are specific in their application areas and constraints.

Tracer techniques are also widely used to study i) the metabolic origin of an element within a compartment ii) the transformations and residence time of

elements in a compartment, iii) the transfer of an element between compartments.

a) **The pulse-labelling of plant shoots** consists of offering $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ or $^{11}\text{CO}_2$ (or $^{15}\text{NH}_4^+$ for studies on N rhizodeposition) to shoots for a short period, once during plant growth. It enables the time resolution between CO_2 (or NH_3) assimilation and rhizodeposition to be determined allowing fluxes of C (or N) through the plant into the soil to be quantified. A series of pulse-labelling can be applied at intervals during plant development to determine changes in C or N allocation in responses to rapid changes in the source-sink relationships in the plant imposed by phenology and/or by the environment.

Basic references

Meharg, A. 1994. A critical review of labelling techniques used to quantify rhizosphere carbon-flow. *Plant and Soil* 166: 55-62.

Merbach, W.; Schulze, J.; Richert, M.; Rocco, E.; Mengel, K. 2000. A comparison of different N-15 application techniques to study the N net rhizodeposition in the plant-soil system. *Journal of Plant Nutrition and Soil Science* 163: 375-379.

Minchin, P.E.H.; McNaughton, G.S. 1984. Exudation of recently fixed carbon by non-sterile roots. *Journal of Experimental Botany* 35: 74-82.

Swinen, J.; Van Veen, J.A.; Merckx, R. 1995. Root decay and turnover of rhizodeposits in field-grown winter wheat and spring barley estimated by ^{14}C pulse-labelling. *Soil Biology and Biochemistry* 27: 211-217.

Warembourg, F.R.; Estelrich, H.D. 2000. Towards a better understanding of carbon flow in the rhizosphere: a time dependent approach using carbon-14. *Biology and Fertility of Soils* 30: 528-534.

Application areas

The technique can be applied in controlled environments or in the field (especially when stable isotopes are used). High temporal resolution: pulse-labelling is relevant to assess rapid changes in fluxes, that is not possible with continuous labelling.

Problems, constraints, do's and don'ts

The use of ^{11}C is limited due to its small half time. Moreover, very few labs in the world have actually the possibilities to produce ^{11}C .

Here are the main constraints in the use of artificial labelling of plants (valid for pulse and continuous labelling) :

- hermetic separation of roots from shoots, especially when the measurement of rhizosphere respiration is required, and to avoid $^{14}\text{CO}_2$ fixation by the enzyme Phospho-Enol Pyruvate Carboxylase in roots;
- regulation of climatic (CO_2 , temperature, hygrometry) and labelling (ratio $^{14}\text{C}/^{12}\text{C}$, $^{13}\text{C}/^{12}\text{C}$) parameters during labelling. Those points are crucial, especially in the case of studies on C allocation to rhizosphere. Despite their importance, they are not always regulated;
- cost of equipments for labelling and sample analyses, isotopes' cost, radioprotection and elimination of wastes (^{14}C).

In addition to those conditions, accurate sampling of the different soil compartments (especially collection of soil adhering to roots exempt of root tissues; see **chapter 1.3.**) is a prerequisite for rhizosphere studies.

Related method sheets

ID	32_Balesdent_a
Parameter	Nature and dynamics of rhizodeposited organic carbon
Soil type	any
Plant species	any
System	mesocosms (plant-soil systems transportable to the laboratory)
Method	Compound-specific stable isotope tracing

ID	32_Dennis
Parameter	^{14}C pulse labelling and allocation imaging
Soil type	All soils
Plant species	All species
System	Rhizoboxes
Method	^{14}C pulse labelling of plant material and ^{14}C allocation imaging using storage phosphor screens

ID	32_Kuzyakov
Parameter	Rhizodeposition; C input by plants into the soil
Soil type	unimportant
Plant species	mainly grasses, unimportant, not trees
System	microcosm, can be used under field conditions
Method	¹⁴C (¹³C) labeling

ID	32_Minchin
Parameter	Short-term carbon release from roots – either as gas or solute
Soil type	any
Plant species	any – but must have moderately fast phloem transport to the roots
System	hydroponic, soil
Method	¹¹C tracer experiment

ID	32_Robin
Parameter	¹⁴C assimilate allocation to rhizosphere compartments
Soil type	All kinds
Plant species	Various cultivated species including maize, ryegrass, <i>Medicago</i> sp.
System	field soil or artificially manipulated, in microcosms of different size
Method	¹⁴C-labelling of plant shoots, ¹²C-CO₂ and ¹⁴C-CO₂ monitoring

ID	32_Warembourg_a
Parameter	Rhizosphere C partitioning using ¹⁴C
Soil type	Artificial substrate, carbonate free soils
Plant species	Any, may be limited by the size of labelling chamber
System	Pots, monoliths in greenhouses and/or laboratory
Method	Short term labelling of plants with ¹⁴CO₂

ID	32_Warembourg_b
Parameter	N₂ fixation using ¹⁵N₂
Soil type	Artificial substrate, Soil
Plant species	N ₂ fixing plant-microbial associations
System	Pots in greenhouses and/or laboratory
Method	Exposure of plant roots to ¹⁵N₂

ID	32_Warembourg_c
Parameter	Rhizosphere C partitioning using ¹⁴C: Rhizosphere respiration
Soil type	Artificial substrate, soil
Plant species	Any plant
System	Pots in greenhouses and/or laboratory
Method	Separation of above and below-ground atmospheres and collection of CO₂

b) **Continuous labelling of plant shoots** consists of offering ¹⁴CO₂ or ¹³CO₂ to shoots for long periods (from first leaf emergence to sampling). In this case, cumulative data are obtained and C

budgets can be established. A combination of different tracer techniques (¹³C/¹⁵N or ¹⁴C/¹⁵N) or the dual labelling of the same element (¹³C and ¹⁴C) is an interesting field to make progress on understanding the plant-soil-micro-organisms interactions and the fate of organic matter.

Basic references

Johansson, G. 1993. Carbon distribution in grass (*Festuca pratensis* L.) during regrowth after cutting – utilization of stored and newly assimilated carbon. *Plant and Soil* 151: 11-20.

Kuzyakov, Y.; Domanski, G. 2000. Carbon input by plants into the soil. Review. *J. Plant Nutri. Soil Sci.* 163: 421-431.

Warembourg, F.R.; Paul, E.A. 1973. The use of ¹⁴CO₂ canopy techniques for measuring carbon transfer through the plant-soil system. *Plant and Soil* 38: 331-345.

Application areas

The method is appropriate for the separation of root-derived and SOM-derived CO₂ and for the estimation of the amount of C transferred by the plant into the soil. This method has poor temporal resolution and therefore it is not appropriate to reveal rapid changes in C fluxes imposed by plant development or by the environment. However, continuous labelling can be applied to field grown plants in undisturbed cores transported to the laboratory and to a canopy technique to label plants in the field.

Problems, constraints, do's and don'ts

Such techniques are difficult to apply in the field due to difficulties to regulate the labelling and plant growth conditions.

Related method sheets

ID	32_Robin
Parameter	¹⁴C assimilate allocation to rhizosphere compartments
Soil type	All kinds
Plant species	Various cultivated species including maize, ryegrass, <i>Medicago</i> sp.
System	field soil or artificially manipulated, in microcosms of different size
Method	¹⁴C-labelling of plant shoots, ¹²C-CO₂ and ¹⁴C-CO₂ monitoring

c) **The addition of labelled solid substrate to the soil** allows to trace its fate in the soil-micro-organism-plant continuum. The transformations (or the degradation) of the substrate into the soil and/or its uptake/utilization by soil micro-organisms or by plants can be studied.

The organic matter or molecule can be either labelled on a single element (ex : ¹³C, ¹⁴C or ¹⁵N) or dual labelled with ¹³C & ¹⁵N.

Basic references

Hodge, A.; Campbell, C.D.; Fitter, A.H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 413: 297-299.

Perelo, L.W.; Munch, J.C. 2005. Microbial immobilisation and turnover of C-13 labelled substrates in two arable soils under field and laboratory conditions. *Soil Biology Biochemistry* 37: 2263-2272.

Application areas

Short term and long term studies to investigate in all kind of soils, planted or not, the metabolic origin of a substrate within a compartment, the transformations and residence time of substrates in a compartment, the transfer of a substrate between compartments. Applicable in microcosms (stable or radioactive isotopes) or in the field (easier with stable isotopes due to rules and regulations applied when radio-isotopes are used in the field).

Problems, constraints, do's and don'ts

There are at least two prerequisites for a such labelling procedure : i) the size of the pool (or compartment) studied should

not change when the labelled substrate is supplied to this pool (e.g.: ¹⁴C-glucose used to trace the pool of soluble sugars) and ii) the pool (or compartment) to be labelled for tracing needs to be homogeneously labelled.

To obtain an accurate picture of microbial decomposition very short time incubation periods are required after the addition of the labelled substrate or the decomposition period is missed. For long-term transformation and transfer studies, the substrate needs to be labelled enough in order to avoid the detection limits due to the dilution of the tracer in the successive compartments within which it is incorporated.

Related method sheets

ID	32_Hodge
Parameter	Plant and AM colonised plant N capture from organic patches dual-labelled with ¹³C and ¹⁵N
Soil type	Loam soil
Plant species	Various grass species, <i>Plantago</i>
System	field soil, microcosms and temporally/spatially heterogeneous substrates (patches)
Method	Various microcosms

ID	32_Nguyen
Parameter	Utilisation of ¹⁴C-glucose by rhizosphere microorganisms in relation to their overall activity
Soil type	Agricultural and forest soils (pH range tested 4-7)
Plant species	maize, ryegrass, <i>Medicago</i> , Birch
System	Field and microcosm soil samples
Method	¹⁴C-glucose assay

d) **The natural abundance method** is based on the natural discrimination of ¹²C vs ¹³C or ¹⁴N vs ¹⁵N isotopes, as a result of isotope fractionation in physical, chemical and biological processes. For C, it occurs during the CO₂ assimilation by the plants. The method needs to grow C4 plants on a C3 soil or *vice versa*. The flux of organic compounds from plant origin are estimated from the $\delta^{13}\text{C}$ value of soil's organic matter pool or soil C respiration. For N, $\delta^{15}\text{N}$ can give insights into N cycle rates or give measurements of N-fixing activities.

Basic references

Balesdent, J.; Balabane, M. 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biology Biochemistry* 28: 1261-1263.

Högberg, P. 1997. ^{15}N natural abundance in soil-plant systems. *Tansley review n°95*. *New Phytologist* 137: 179-203

Rochette, P.; Flanagan, L.B. 1998. Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Sci. Soc. Am. J.* 61: 466-474.

Application areas

The method is mainly devoted to quantify in the field the long-term contribution of root-C to soil C. It is applicable in the field, without special equipments, as the labelling occurs naturally. The main limitation is the low temporal resolution that does not allow its use for short-term studies on rhizosphere processes.

Problems, constraints, do's and don'ts

Sample analyses require very sensitive and thus expensive mass spectrometers. Another constraint is to identify soils cropped with C3 or C4 plants for a long period (for C budget studies). There are some difficulties to interpret the variation in $\delta^{15}\text{N}$ in soil-plant systems (isotopic source effects vs fractionation).

Related method sheets

ID	32_Balesdent_b
Parameter	Root-derived carbon: amount, quality
Soil type	Any
Plant species	C4-plant in C3 environment or vice-versa
System	In the field
Method	Natural ^{13}C signature of C4 plants in the field

ID	32_Gioacchini
Parameter	Soil Total Organic Carbon and $\delta^{13}\text{C}$
Soil type	All types of soil
System	e.g. field soil, microcosm
Method	Elemental analysis and Continuous flow-isotope ratio mass spectrometry (CF-IRMS)

3.2.3. Isotope-based detection devices

Liquid scintillation and gamma counters have been widely used to detect radioactivity for years. The imaging of β -radiation by electronic auto-radioagraphy is appropriate for a wide range of radioactive isotopes, showing the pattern of element distribution from the shoots to the roots and mycorrhizal web.

Very sensitive detection devices for stable isotopes, such as Nuclear Magnetic Resonance (NMR), Gas Chromatography (GC)-Mass Spectrometry (MS), GC-Isotope Ratio MS, are now available, allowing quantitative and qualitative *in situ* analyses of the C and N composition of rhizosphere samples. This area is promising to identify and localize the root released compounds, to estimate their residence time and to assess the transfer of elements between compartments and their dynamics within a given compartment (capture of elements by microorganisms and plants from labelled patches in soil).

Basic references

Derrien, D ; Balesdent, J. ; Marol, C. ; Santaella, C. 2003. Measurement of the $^{13}\text{C}/^{12}\text{C}$ ratio of soil-plant individual sugars by gas chromatography/combustion/ isotope-ratio mass spectrometry of silylated derivatives. *Rapid Communications in Mass Spectrometry* 17: 2626-2631.

Fitter, A.H.; Graves, J.D.; Watkins, N.K.; Robinson, D.; Scrimgeour, C. 1998. Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. *Functional Ecology* 12: 406-412.

Hodge, A.; Campbell, C.D.; Fitter, A.H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413: 297-299.

Slater, R.J. (ed.) 1990. *Radioisotopes in Biology. A practical approach*. Oxford University Press, New York, 307 pp.

Application areas

All kind of soils. Universal use of β -radiation counters and high sensitivity.

Detection of root released compounds by GC-MS can be done from adhering soil of plants grown in microcosms or field grown plants (previously labelled with ^{13}C).

Problems, constraints, do's and don'ts

The imaging of β -radiation by electronic auto-radiography is limited to upper layers of flat microcosms or rhizotrons, due to poor energy emitted from some isotopes (^{14}C). An artefact can be caused by the preferential growth of roots and mycorrhizal network near the window of the rhizotron.

Compound-specific analyses require very sensitive and therefore expensive detectors (mass spectrometers, NMR..). Non-volatile (polar) compounds need derivatization prior to GC.

Related method sheets

ID	32_Balesdent_a
Parameter	Nature and dynamics of rhizodeposited organic carbon
Soil type	any
Plant species	any
System	mesocosms (plant-soil systems transportable to the laboratory)
Method	Compound-specific stable isotope tracing

ID	32_Balesdent_b
Parameter	Root-derived carbon: amount, quality
Soil type	any
Plant species	C4-plant in C3 environment or vice-versa
System	In the field
Method	Natural ^{13}C signature of C4 plants in the field

ID	32_Dennis
Parameter	^{14}C pulse labelling and allocation imaging
Soil type	All soils
Plant species	All species
System	Rhizoboxes
Method	^{14}C pulse labelling of plant material and ^{14}C allocation imaging using storage phosphor screens

ID	32_Finlay
Parameter	Distribution of radioactive isotope tracers in the mycorrhizosphere
Soil type	acid forest soils
Plant species	Pine, Birch, Spruce
System	field soil or artificially manipulated, spatially heterogeneous substrates
Method	Electronic autoradiography of flat laboratory microcosms

ID	32_Gioacchini
Parameter	Soil Total Organic Carbon and $\delta^{13}\text{C}$
Soil type	All types of soil
System	e.g. field soil, microcosm
Method	Elemental analysis and continuous flow-isotope ratio mass spectrometry (CF-IRMS)

ID	32_Hacin
Parameter	Photosynthate partitioning to root and nodule meristems
Soil type	Plant nutrient solution
Plant species	Soybean
System	Plastic growth pouch, Split-root system (see 11_Hacin)
Method	Visualization of ^{14}C labelled root and nodule meristems by Eriochrome black staining and autoradiography

ID	32_Schweiger
Parameter	Stable C and N isotopes in mycorrhizal research
Soil type	any
Plant species	mycorrhizal
System	None specified
Method	Isotope Ratio Mass Spectrometry (IRMS)

3.2.4. Non isotope-based techniques

Genetically modified bacteria are used as biosensors to report on the incorporation of an element coming from a given compartment. The expression of reporter genes that is proportional to the availability of a substrate allows the spatial localisation and the quantification of compounds in the laboratory. Example: organic compounds released by the living roots. The reporter gene can be specific of a substrate or calibrated to a range of compounds.

It is applicable in real soils in laboratory experiments or in simplified hydroponic systems. The use of a biosensor is more suitable at the single root scale than at a large spatial scale. The relation between biosensor signal and substrate availability needs a calibration, prior to the experiments.

Basic reference

Yeomans, C.V.; Porteous, F.; Paterson, E.; Meharg, A.A.; Killham, K. 1999. Assessment of lux-marked *Pseudomonas fluorescens* for reporting on organic compounds. FEMS Microbiology Letters 176, 79-83.

Problems, constraints, do's and don'ts

Field experiments are usually not possible: the GMO are restricted to controlled, restricted areas of authorized laboratories.

In real soils, the biosensor targeted to reveal a specific compound (specificity) respond also to this compound whatever its origin.

Related method sheets

ID	32_Paterson
Parameter	C-flow from plant roots
Soil type	Sand
Plant species	Broad range
System	Microcosm
Method	Biosensor reporting

ID	32_Standing
Parameter	Carbon exudation from plant roots
Soil type	Sandy loams to clays
Plant species	Developed on <i>Triticum aestivum</i> cv. Scout (wheat)
System	Liquid batch culture, soil slurries, soil microcosms using field soils
Method	Carbon biosensing with <i>lux</i>-marked <i>Pseudomonas fluorescens</i>

3.2.5. Conclusion

The current development of techniques presented in this chapter represents a great potential to understand the element transfer and turn-over in the plant-soil-microbes continuum. The improvement of detection sensitivity and reliable micro-samplings in the rhizosphere are still stimulating challenges. Another challenge is to assess the element cycling and transfer in the soil food webs, the rhizosphere being a biological reactor stimulating these multiple biotic interactions in the soil.

In the case of plant C, a promising aspect is the combination of tracer and molecular approaches allowing to measure the incorporation of C into soil RNA and DNA pools (stable isotope probing: as an example, see **43_Prosser_b**). This is an elegant and pertinent way to determine the 'active' rhizosphere community, heterotroph as regard to rhizodeposited C. This is one of the steps to access rhizosphere functions.