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Tracing the transfer of recently assimilated carbon into the soil after *in situ* $^{13}CO_2$ pulse labelling of trees

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ABSTRACT

The fate of carbon in the soil plant system was followed by using pulse-labelling of 10 m tall trees in the field with $^{13}CO_2$ for a short period of time. The study is conducted on three tree species (beech, oak and pine) that are among the major species in European forests. Trees are labelled at three distinct phenological phases during the growing season. The assimilated ^{13}C by plants during the pulse labelling was then tracked in soil CO_2 efflux with a high temporal resolution using tuneable diode laser absorption spectrometry and in the microbial compartment from soil cores (root + mycorrhiza transfer) and in mesh cores that permit the ingrowth of mycorrhiza hyphae but exclude roots (no-root cores).

KEYWORDS: carbon allocation, *Fagus sylvatica*, *Pinus pinaster*, *Quercus petraea*, residence time, soil CO_2 efflux

1. INTRODUCTION

Ecosystem respiration almost balances photosynthetic carbon assimilation in forest ecosystems, (Granier et al. 2000) and the two-third of the sources are located belowground (Janssens et al. 2001). These sources include roots and associated symbiotic organisms like mycorrhiza hyphae, rhizosphere microbes that feed on root exudates, and saprotrophic bacteria and fungi that feed upon litters and soil organic matter. The importance of carbon transfer to the rhizosphere through roots and mycorrhiza has been recognized (Högberg and Read 2006). A precise partitioning of these fluxes requires a better understanding of carbon residence time in these compartments and

of carbon allocation among them. The recent development of tuneable diode laser absorption spectrometry (TDLAS) allows *in situ* simultaneous measurements of effluxes of $^{13}CO_2$ and $^{12}CO_2$ at a high frequency, and it can be used for tracking ^{13}C in respiratory fluxes during the chase period after pulse labelling (Bahn et al. 2009).

The aim of the project is to track and quantify the assimilated ^{13}C carbon in soil CO_2 efflux by TDLAS and in the microbial biomass and bulk soil collected in soil cores (root + mycorrhiza transfer) and in mesh cores that permit the ingrowth of mycorrhiza hyphae but exclude roots (no-root cores). The study was conducted on three tree species (beech, oak and pine) at three distinct phenological stages during the growing season.

2. MATERIALS AND METHODS

2.1. Sites and trees

The study was conducted on beech (*Fagus sylvatica*) in the state forest of Hesse (48°40′40″N, 7°04′05″E, 300 m elevation), on oak (*Quercus petraea*) in the state forest of Barbeau (48 ° 28’ N, 2° 46’ E, elevation 92 m) and on pine (*Pinus pinaster*) in the INRA domain of Pierroton (44° 45’ N, 0° 42’ W, elevation 60 m). For each labelling date three trees were selected (8-10 m tall trees). Two of these trees were selected for labelling (LT) and one tree was selected as a control (CT). One year before labelling date, trenches have been dug around each tree, further lined with a polyethylene film and filled back. The root system of each tree has been then confined in a soil volume. We assumed that roots exudates within this area are therefore coming from the isolated tree, and all roots and root exudates of this tree only occurred in this volume.

2.2. Crown labelling chambers

Scaffolds were built around labelled trees in order to set up the chamber at the top of the tree (Fig 1). The crown labelling chamber was made of 200 µm polyane film and the base of the chamber (1 m^2) was made with two stainless steel or PVC half- plates with circular openings to accommodate the trunk and the cooling device tubes The volume of the labelling chamber ranged from 20 m^3 to 40 m^3 according to the size of the crown. Air temperature, air humidity and photosynthetic active radiation inside the labelling chamber were recorded. The chamber air was controlled and maintained at the temperature of the outside air.

Figure 1: Labelling chamber in Hesse forest (Sept. 2008)

2.3. Labelling

The labelling chamber was closed between 9:00am and 10:00am UT. Evolution of both $^{12}CO_2$ and $^{13}CO_2$ concentrations inside the chamber were monitored simultaneously with a $^{12}CO_2/^{13}CO_2$ infrared gas analyser (S710, SICK/MAIHAC). 25 L of pure $^{13}CO_2$ (99.299 atom %, Eurisotop) were injected into the labelling chamber. The CO2 concentration inside the chamber was monitored and adjusted around ambient concentration (c.a. 400 ppmv). After 2 to 4 hours, the gas cylinder was emptied and the labelling chamber was opened and removed.

2.4. Soil CO_2 efflux and ^{13}C composition

Soil CO_2 effluxes (FS) and it isotope composition ($\delta^{13}C_{FS}$) were measured by TDLAS with a trace gas analyzer (TGA 100A) coupled to flow-through chambers (Marron et al. 2009). A manifold was used to switch between working standards and the chamber inlet and outlet lines. A second manifold was used to switch between chamber inlets and outlets, allowing each chamber to be measured every 40 to 50 minutes. The soil chambers were made of stainless steel and allowed the enclosure of 314 to 380 cm^2 of soil. Soil CO_2 efflux and the isotope composition of soil CO_2 efflux were calculated according to Marron et al. (2009).

2.5. Soil cores

Four soil cores were taken three months before labelling and put intact into nylon mesh bag (30 µm) allowing ingrowth of fungal hyphae but excluding ingrowth of roots. These root exclusion cores were re-sampled 3 and 6 days after labelling. Additional soil cores (two per tree and per dates) were collected at several dates during the two weeks following labelling. Root-free soil was incubated in a closed chamber for determining the isotope composition of respired CO_2, stored at 4°C for less than a week and used for estimation of microbial biomass. An aliquot of soil was dried, ground into powder and used for determination of $\delta^{13}C$ of bulk organic matter. Microbial biomass was estimated using the fumigation-extraction method. Isotope composition of both chloroform-fumigated and non-fumigated extracts were determined and used to calculate the isotope composition of the microbial biomass.

3. RESULTS AND DISCUSSION

Labelling were done in Sept 2008 and May 2009 on beech, May 2009 on oak and June 2009 on pine, and other labellings are foreseen during summer 2009 (beech, oak and pine) and winter (pine). A 10‰-enrichment in $\delta^{13}C_{FS}$ of LT compared to control was observed 20 to 30 hours after the beginning of the labelling (Fig 2). This time lag was lower than those previously reported in 3-4 m tall trees (Carbone et al. 2007; Högberg et al. 2007), and it could be ascribed to a better time resolution in the TDLAS-based measurements..

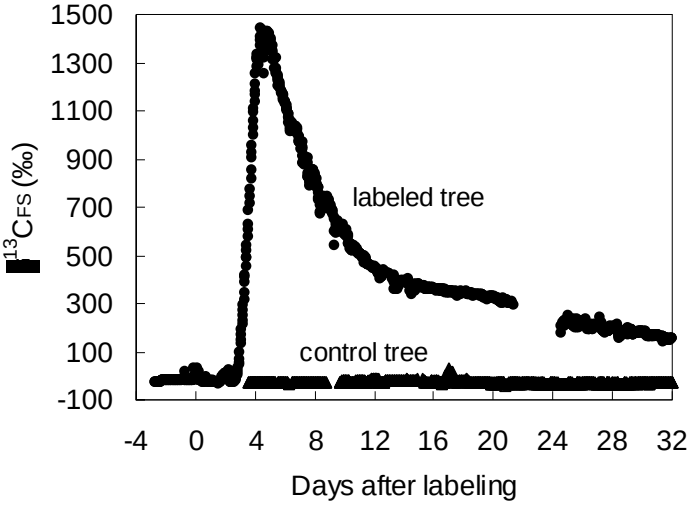

Figure 2: Time courses of the isotope composition of soil CO_2 efflux ($\delta^{13}C_{FS}$, ‰) after labelling a beech tree in Hesse forest in September 2008

The maximum labelling in the microbial biomass was observed 2 to 3 days after labelling, indicating a fast transfer of photosynthate to the microbial community, with almost no ^{13}C detectable in the bulk soil or in non fumigated soil extracts (Fig 3).

Transfer of soil carbon is also observed in root exclusion cores highlighting the fact that not only roots but also mycorrhiza are involved in the transfer of C into forest soils (Fig. 4). These findings reinforced previous results showing a close coupling between photosynthesis and soil CO_2 efflux (Knohl et al. 2005).

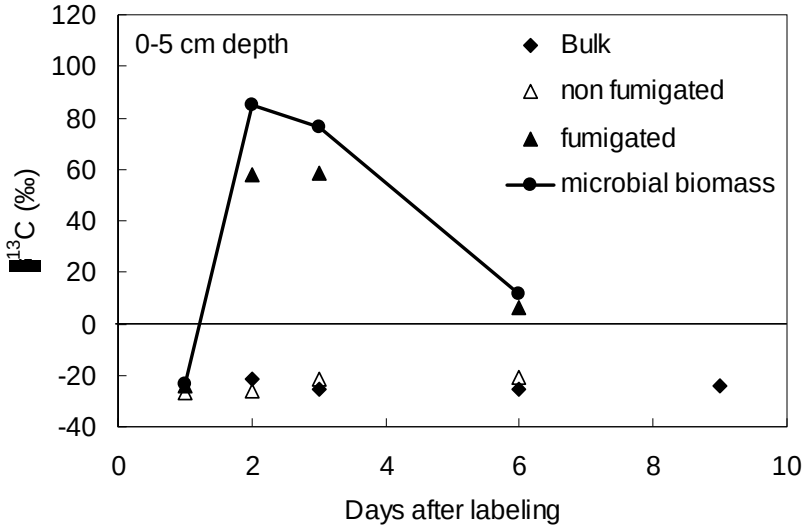

Figure 3: Isotope composition of bulk soil, non fumigated and fumigated soil extracts, and microbial biomass after labelling a beech tree in Hesse forest in Sept. 2008

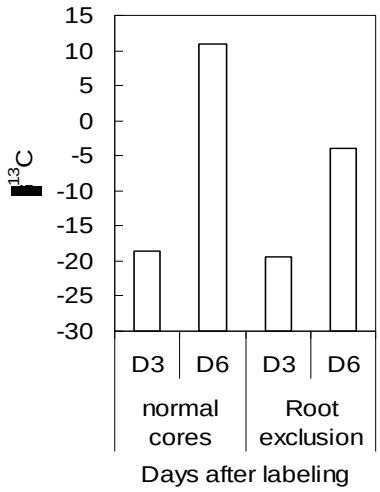

Figure 3: Isotope composition of CO_2 respired by soil from normal cores or root exclusion cores after labelling a beech tree in Hesse in May 2009

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