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> Interactive Comment

# Interactive comment on "Carbon transfer, partitioning and residence time in the plant-soil system: a comparison of two <sup>13</sup>CO<sub>2</sub> labelling techniques" by M. S. Studer et al.

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We would like to thank Referee #2 for the comments on the manuscript. It helped us to see on which issues we have to be clearer to prevent misunderstandings.

1) Objective of the study

The focus of this study was not to develop a new (sophisticated) approach of modelling different C pools (to estimate C residence times), but to perform a direct method comparison of the two main 13CO2 labelling techniques regarding the three aspects C transfer, partitioning and residence time. We used the same experimental designs and applied (commonly used) simple models / approaches. We do not aim for providing



new mathematical tools for data analysis, but for highlighting the applicability of the existing (simple) models / approaches to assess these three aspects. We agree with Referee#2 that the estimation of the residence time (3rd aspect) of specific pools would require a more sophisticated modelling than performed in our experiment, since the plant-soil system was not in a steady state (P16254 L20-22, P16257 L3-5). However simple decay models are often applied to gain information on turnover or residence time in tracer studies (e.g. in Butler et al., 2004; Carbone et al., 2007; Högberg et al., 2008) and in this study we would like to highlight the difficulties related to it.

2) Too low sampling frequency (for complex modelling)

This issue has already been partly discussed in the answer to Referee#1 (Section 3). Destructive sampling of entire plant-soil systems to assess the allocation of label added in different compartments/pools is laborious and therefore mostly limited to a few sampling dates (especially when a holistic approach is chosen, i.e. many different compartments are analysed). We've performed destructive samplings at five dates and separated seven compartments (240 solid samples). In addition we sampled the soil respiration at 12 (PL) and 14 (CL) sampling dates (234 gaseous samples) and both samplings have been performed with higher sampling frequencies at the beginning of the experiments. This sampling frequency is comparable with other tracer studies, where decay models have been applied to assess the residence/turnover time (Butler et al., 2004; Carbone et al., 2007; Högberg et al., 2010, 2008; Keel et al., 2012; Rangel-Castro et al., 2005; Ruehr et al., 2009; Streit et al., 2012; Warren et al., 2012).

3) Improving the models to account for growth (non-steady state)

Referee#2 recommends using a non-zero asymptote for the exponential function to model the 13C dynamics after pulse labelling and a non-constant a-parameter for the logistic function in the CL to account for growth (incorporation of the label into the structural pool). As mentioned above (section 1) it was not our aim to find the best model fit, but to apply the commonly used models for data analysis and compare and

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critically discuss the results obtained by the two labelling techniques. Nevertheless we tested the model fit according to the suggestions of Referee#2 by using the formula y =  $(a-c)^*e^{(-k^*(t-b))+c}$  for the PL data and y =  $(a + c^*t) / (1 + e^{(-k^*(t-b))})$  for the CL data. Fitting another unknown parameter was not possible for compartments with a late peak appearance (due to low number of sampling dates after the peak). Yet, we calculated it for the leaves, petioles, stems and the soil respiration (Figure 1 below). Applying the above mentioned functions resulted in largely different mean residence times (MRTs) and higher model accuracies (increased R2, decreased RMSD) in the PL, while it did not affect the results of the CL much (Figure 2 below). The MRTs estimated were much shorter than with the common decay functions, whereas the MRT estimates of the CL technique were slightly higher (in most compartments) compared to the estimates obtained by the simple logistic function (steady state assumption). Thus, including a parameter "c" into the functions, accounting for non-steady state (e.g. plant growth), resulted in comparable MRTs for the two labelling techniques (MRT around 1 day). These results confirm our hypotheses i) that the simple exponential decay model (PL) overestimates (P16254, L21-22) and that the simple logistic model underestimates (P16255, L4-7) the actual MRTs, when applied to a system at growth and ii) that the logistic model of the CL is more robust to estimate the MRT than the exponential model (P16255, L3-4). (Specific comment on the model fits: The parameter "c" introduced into the logistic function (CL) is conceptionally thought to be positive when a system is at growth, leading to a steady increase over time as shown in the revised Figure 1 (see reply to Referee#1). The model fits of our data returns negative c-parameters (e.g. in petioles). This is probably due to the fact that our experiment was too short to detect the slow accumulation of the label into the structural pool (second accumulation phase, revised Figure 1) and due to large interplant variability.)

Further replies to the specific comments of Referee#2

a) Transfer time

Referee#2 pointed out the misuse of the word "velocity", which we will correct in the

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revised version of the manuscript. We agree with the Referee#2 that the signal peak is not only related to the transfer time, but to the balance between import and export (as stated in P16248 L16-19). In the method description the definition might be difficult to understand, but in the discussion we state how the "mean transfer time" can be interpreted for the two labelling techniques (P16251, L18-21 and P16251, L27 - P16252 L2). Further we are aware, that transfer time accounts for the mixing with an unlabelled pool (P16250 L14-17), what leads to a faster minimum transfer time (estimated by the first signal detection) observed with PL (higher label strength of assimilates) than with CL (P16251 L8-11). Thus the transfer times estimated with the two techniques are not directly comparable (what will be mentioned in the revised manuscript more clearly).

b) C partitioning estimations based on the amount of 13C at the signal peak

We are aware that the amount of 13C at the peak is not equal to the total amount of 13C assimilated. We assessed the partitioning patterns by the amount that remained in the system at a certain point in time (P16248 L28 - P16249 L3). As an alternative to this approach we tested if one could also use the model parameters, i.e. the amount at the signal peak or the stationary phase ("a"), to estimate the partitioning (P16249 L3-L5). This approach would have the advantage that it is independent on the sampling date, but it is closely related to the sampling frequency. And as stated in the manuscript we might have missed the "real" peak (P16254 L4-L7), due to low sampling frequency, wherefore this alternative approach is not suited to estimate the C partitioning (at least in this experiment). A further option would be to calculate the partitioning based on the parameter "c" (asymptote in PL), as suggested by the Referee#2. Unfortunately we cannot test this approach with our data, since "c" cannot be fitted for all plant-soil compartments.

c) Which parameters (a, b) are fitted?

In the logistic model (CL) parameters a, b and k were fitted. For the exponential model (PL) different ways of applying the function  $y=a^*e^*(-k^*t)$  exist in literature and often it

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is not described how exactly the fit was made (e.g. which parameters are fitted and how). Conceptually "t" refers to the time after the label peak (sometimes set equal to the time after labelling) and "a" refers to the amount at the label peak. If there is a time lag in the peak (i.e. the time after labelling is not equal to the time after the label peak) the formula can also be expresses as  $y = a^{*}e^{(-k^{*}(t-b))}$ , whereas "t" refers to the time after pulse labelling and "b" to the time at the label peak. The problem is that a high sampling frequency is needed to detect the "real" peak time and amount (as indicated by Referee#2), but as discussed above (section 2) the sampling frequency is often limited. There are four theoretical options to apply the model: 1) set "a" and "b" constant, 2) fit "a" and "b", 3) fit "a" and set "b", 4) set "a" and fit "b". Conceptually we think that option 3 and 4 are not correct, since "a" and "b" are linked with each other (parameters of the label peak) and should therefore be considered both as known (option 1) or both as unknown (option 2) in the model. The problem of option 2 is that "b" can not be fit by the exponential decay model itself (which describes only the decay after the peak), but would require a more complex model to describe the 13C dynamics including the initial signal increase before the peak. Therefore we decided to set "a" and "b" (option 1) based on the peak observed in our data (amount measured at the specific sampling date). Option 3 (fitting "a" without fitting "b") has often been applied in literature too. In our case option 3 results in slightly higher R2 compared to option 1 in leaves (0.93 vs. 0.90) and petioles (0.94 vs. 0.93), but not in the other tissues and it does not change the estimated mean residence times to a large extend (max. 3 days). A much greater effect has the application of a non-zero asymptote (as discussed above in section 3).

d) Standard errors of model estimates?

There is no standard deviation to give for the models, since the three sampling points at each sampling date do not represent the same plant individuals, but each point is a distinct individual (thus the model was not fitted three times). As an indicator for the accuracy of the model fits we stated the R2 and the RMSD (based on the difference

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between the measured and the predicted values).

We thank Referee#2 for all other specific comments not commented here. They will be included in a revised version of the manuscript.

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Fig. 1. Figure 2 of the manuscript with the application of the exponential and logistic model fit

including a parameter c to account for growth (non steady state assumption).

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Fig. 2. Mean residence time (MRT), coefficient of determination (R2) and root mean square	е
deviation (RMSD) obtained by exponential or logistic models with/without the assumption of	сf
steady state.	

 $\mathbf{R}^2$ 

steady

0.90

0.93

0.92

0.92

0.62

0.73

0.84

0.94

0.92

0.87

0.87

0.79

0.57

0.69

0.84

non-

steady

0.97

0.94

0.93

0.84

0.94

0.93

0.88

0.87

0.60

0.69

0.84

RMSD [mg]

steady

2.6

0.1

0.7

0.2

0.2

0.01

0.1

3.1

0.4

1.8

0.3

0.3 0.03

0.2

0.1

non-

steady

1.5

0.1

0.7

0.1

3.1

0.4

1.8

0.3

0.03

0.2

0.1

MRT [days]

steady

3.5

21.1

13.0

8.5

34.0

5.6

1.9

1.2

0.9

1.1

1.3

0.8

0.9

0.8

1.4

a) Continuous labelling

non-

steady

1.0

1.8

0.5

1.7

1.4

1.2

1.4

1.1

1.2

0.7

1.4

Model

assumption

Petioles

Cuttings

Stems

Roots

MB

SOM SR

Leaves

Petioles

Stems

Roots

SOM

MB

SR

Cuttings

a) Pulse labelling Leaves