

***Interactive comment on “Short-term natural  $\delta^{13}\text{C}$  variations in pools and fluxes in a beech forest: the transfer of isotopic signal from recent photosynthates to soil respired  $\text{CO}_2$ ” by O. Gavrichkova et al.***

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We thank the anonymous referee for the detailed comments, for signed uncertainties in the interpretation of the results and in general for positive evaluation of the manuscript.

We have considerably modified the section dedicated to interpretation of soil respiration data also respect to the proposed by the referee explanation for the observed patterns in  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  due to non-biological processes and sampling-induced problems. More data was also added on  $^{18}\text{O}$ : we have analysed  $^{18}\text{O}$  signature in PSS

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and studied its relation to environmental parameters coupled to transpiration efficiency. The day/night variation here was much more pronounced than in  $^{13}\text{C}$  PSS. We have incorporated almost all requested changes, in particular:

# I do not see much diurnal variability in the carbon isotope ratio leaf sugars, and no strong repeated pattern. The standard error bars mostly overlap throughout the time series, and where there are times that appear to possibly show a difference, patterns of variability at the different canopy levels are out of phase. The measures of variance in the plotted time series (SEM bars) do not appear to get propagated through the subsequent correlation analysis. I am more inclined to argue that you have three, different but flat, time series of leaf sugar isotope ratios, with a small amount of irregular noise (and the same for phloem sugars). This is apparent in the different scales needed to see peaks on Fig. 6. At most, there is only 1 per mil diurnal variability in leaf sugars at any one canopy level, with offsetting patterns across canopy layers. This means that diurnal variability in photosynthetic discrimination, to the extent that it is reflected in variability in the isotope ratio of leaf sugars and phloem sugars, does not explain the degree of isotopic variability you see downstream in soil respiration (several per mil).

We agree that diurnal variability found in LSS  $^{13}\text{C}$  was weakly pronounced. However, other papers, dealing with LSS day/night variation reported similar range in  $^{13}\text{C}$  values, where day/night variation doesn't exceed 1-2‰ (Kodama et al., 2008, Ghashgae et al., 2001, Rascher et al., 2010 (PSS), Brandes et al., 2006, Gessler et al., 2007, 2008). In our case 12h periodicity of LSS  $^{13}\text{C}$  was found in single replicate trees, which were however quite different between each other in absolute values of LSS  $\delta^{13}\text{C}$ . By mean, day-night range was 1.4 ‰ here. We however agree that the link between respired  $^{13}\text{CO}_2$  and  $\delta^{13}\text{C}$  in LSS and PSS was overestimated in the text. We have modified this part.

# The observed variability in  $\delta^{13}\text{C}$  of  $\text{CO}_2$  from the soil is probably largely (or even entirely, given your results) due to lack of steady state conditions between  $\text{CO}_2$  production in soil and the surface flux (see Moyes et al 2010). Small, but diurnally variable

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respiration rates, such as shown in Fig. 4, can lead to large variations in isotope ratio of the soil CO<sub>2</sub> flux. When respiratory production decreases as soils cool at night, the flux becomes progressively enriched via a “distillation effect” as <sup>12</sup>CO<sub>2</sub> leaves soil pores faster than <sup>13</sup>CO<sub>2</sub>. And when production increases again the following day when soils warm, <sup>12</sup>CO<sub>2</sub> molecules begin to emerge from soil pores faster and the flux becomes depleted. This can happen with no variability in the isotope ratio of the carbon source being metabolized and respired.

We thank the referee for the proposed explanation for our data patterns. Papers of Moyes and co-authors were overlooked during our literature search. We have interpreted our data in respect to observations and model proposed by Moyes et al. 2010. Magnitude of respiration fluxes and the range of day-night variation goes in accord with the proposed theory on diffusion fractionation determination of diurnal <sup>13</sup>C respiration patterns.

# The approach of linking variability in a leaf sugar pool with a phloem sugar pool, and then a respiratory flux involves many important assumptions that are not addressed. For example, LSS and “recently fixed organic matter” are used interchangeably (Disc. 4.1, line 21). In actuality, a lot of processes could decouple or modify relationships between leaf sugars and downstream pools and fluxes, including leaf respiration, biosynthesis, etc. (see Tcherkez 2007, Ghashghaie et al. 2001, Bowling et al. 2008). Actually, the simple model of photosynthetic fractionation -> leaf sugar pool -> phloem sugar pool -> rhizosphere respired CO<sub>2</sub> could be presented as an oversimplification, and the data could be used to demonstrate that the truth is much more complicated. This may well be the best use of these data. The weighted mean  $\delta^{13}C$  of soluble sugars is probably around -26 per mil, phloem sugars are actually more depleted than this (compare to Hobbie and Werner 2004 and Damesin and Delarge 2003), and the average of soil respiration is enriched to about -21 per mil. These differences are interesting and should be interpreted more.

We thank for this observation. We have specified in the text that our LSS data contain

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information mainly on soluble sugars diurnal dynamic, whereas in the cited literature soluble organic matter was often extracted and analyzed and may differ in its  $\delta^{13}C$  from LSS. We also agree that the proposed model photosynthetic fractionation -> leaf sugar pool -> phloem sugar pool -> rhizosphere respired CO<sub>2</sub> is a simplification, but it is the base of the theory when we attempt to utilize natural abundance technique and other TSA methods for determination of the time lags. We have tried to emphasize throughout the discussion the weak points of this method, associated mainly, as proposed, with interaction of numerous processes while <sup>13</sup>C signature is propagated belowground with phloem flow.

# The three-day period of observations is limited. Others (e.g. Wingate 2010) have shown lag times of up to several days. Discuss the days leading up to the measurement period, and maybe add earlier dates to figure 1, if available.

Yes, we agree that 3 d period is limited. We have specified in the initial manuscript variant that lags no longer than 1.5d could be detected from our data, not excluding therefore that the velocity of carbohydrate transport is probably higher than 1m<sup>h</sup>-1. 24h lag found between LSS and PSS is probably a result of the similar environmental patterns between the adjacent days, which drive C<sub>i</sub>/C<sub>a</sub> ratio. The real time lag is probably an aliquote of 24. We have added the data on <sup>18</sup>O in PSS, and, as suggested, studied its relation to environmental parameters of up to 5 days prior to sampling, improving therefore the estimation of the time lag.

# Water content of 0.19 m<sup>3</sup> m<sup>-3</sup> seems fairly high for a wilting point (unless I am more used to sandy soil and drought-tolerant spp). Figure 1 shows 0.22-0.25.

Near the surface SWC was lower than 0.19 m<sup>3</sup> m<sup>-3</sup>, but along the soil profile was increasing gradually. We propose the hypothesis on midday partial stomatal closure in respect to several evidences: low SWC, high VPD and decrease in photosynthetic activity (GPP, we have added to the 1st figure) close to midday.

# Soil chamber isotope measurements are really hard to do without creating bias (see

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papers by Nickerson, Risk, Kammer, etc.). Closed chambers can suppress fluxes as headspace concentration builds up (Davidson 02). Removing gas from a sealed soil chamber causes advection out of the soil. This can cause incorrect measurements because CO<sub>2</sub> mole fraction in even shallow soil pores is very high and follows different mixing relationship than the evolved CO<sub>2</sub> entering the chamber headspace by diffusion under steady state. 10 mL were pulled five times from the 7 L chamber, which may not seem like much, but that's 50/7000 by volume or ~700 Pa. Pressure perturbations of less than 1 Pa can cause measurement problems (e.g. see Xu et al. 2006). I believe the patterns shown in Fig. 4 do reflect the real patterns in flux rates and isotope ratios, but you should discuss or defend against the possibility of measurement biases in the paper.

We thank for this observation. We used the standard dimensions of soil chambers, often reported in papers where Keeling plot are constructed for δ<sup>13</sup>C evaluation (6-10L). Nowhere this point was discussed actually. We have add to the discussion text possibility of overestimation of the enrichment due to sampling caused biases. At low efflux, as in our site the "contamination" of the respiration with mass flow of air from the soil into the chamber in fact could substantial.

#You might also give an estimate of precision of using IRMS peak areas to get CO<sub>2</sub> mole fraction – I have had to accept some error with this approach compared to other IRGA-based methods.

From our tests, peak areas give reliable estimates of CO<sub>2</sub> concentrations, at least in the concentration range of interest.

# The explanations of causes of isotope variability in both carbon and oxygen of sugars are a bit rushed and inaccurate. It is not the decline in photosynthetic rate that decreases fractionation against <sup>13</sup>CO<sub>2</sub>, but the decline in ci/ca. Starch enrichment is not due to the uneven distribution of <sup>13</sup>C in hexoses, but the tendency for enriched or depleted portions to be processed differently at metabolic branch points (e.g. Tcherkez

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et al. 2004). Differences in VPD and conductance affect variability in δ<sup>18</sup>O by changing relative evaporative enrichment (more evaporation = more enrichment) of leaf water (the Craig-Gordon model, e.g., see Roden 1999), and the Peclet effect is invoked to explain deviation from predicted evaporative effects (Barbour 2001).

We have corrected the inaccurate interpretation of the processes. A separate section was dedicated to δ<sup>18</sup>O. We have added the data on δ<sup>18</sup>O composition of PSS and used it to calculate the velocity of carbohydrate's transport instead.

#Replace all instances of the word "confirm" with "support".

Changed as requested

# "consecutive days" instead of "consequent days".

Changed as requested

# "Shortly" is a synonym for "soon". I think you mean, "In short" for "briefly".

Changed as requested

#First sentence of 2.6: Canopy weighted delta <sup>13</sup>C (not "CO<sub>2</sub>")

Yes, It is a mistake, we have corrected it

#In 3.4 "Soil CO<sub>2</sub>" is CO<sub>2</sub> in the soil pores - I think you mean "The soil CO<sub>2</sub> surface flux".

Changed as requested

#I haven't seen "Meteo" used as a heading before. Maybe use "Meteorological data".

Changed as requested

# "Leaves" as a heading is broad – maybe "Leaf sugars". . .

Changed as requested

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#Y-axis labels on Fig. 2b, right hand side are confusing – 28, 28, 27, 27, 26, 26. . . I think there's a decimal missing (?)

Corrected

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