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***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.***

**Anonymous Referee #3**

Received and published: 31 March 2011

This paper describes an effort to link diurnal variability in the carbon isotope ratio of extractable leaf sugars at three canopy levels of deciduous trees with the variability in  $\delta^{13}\text{C}$  of phloem sugars and soil-respired  $\text{CO}_2$ . Data were collected every  $\sim 3$  hours for a 3-day period, and then transport time lags were inferred from correlation analysis. The results presented are useful, timely, and relevant to the journal, and the special issue in particular. For the most part, the quality of the measurements and experimental design appears to be very good (but see comments). I do, however, have some concerns about some critical aspects of the interpretation of the data, which I

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believe should be addressed before the manuscript is considered ready for publication.

### Data interpretation

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).

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 respiration rates, such as shown in Fig. 4, can lead to large variations in isotope ratio of the soil  $\text{CO}_2$  flux. When respiratory production decreases as soils cool at night, the flux becomes progressively enriched via a “distillation effect” as  $^{12}\text{CO}_2$  leaves soil pores faster than  $^{13}\text{CO}_2$ . And when production increases again the following day when soils warm,  $^{12}\text{CO}_2$  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.

Correlation (direct or lagged) will be high for any two patterns with similar frequencies, which is especially common for diurnal or seasonal variability, regardless of whether or

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not there is any causal link. Please use caution in the way you interpret such instances of correspondence.

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  $\delta^{13}\text{C}$  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.

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.

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.

## Measurements

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

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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  $\sim 700$  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. 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.

### Presentation

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 *c<sub>i</sub>/c<sub>a</sub>*. 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 et al. 2004). Differences in VPD and conductance affect variability in <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).

Replace all instances of the word “confirm” with “support”.

“consecutive days” instead of “consequent days”.

“Shortly” is a synonym for “soon”. I think you mean, “In short” for “briefly”.

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

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”.

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

“Leaves” as a heading is broad – maybe “Leaf sugars”...

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

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**BGD**

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