Response of $\delta^{13}C$ in plant and soil respiration to water pulse

Response to Referee #2

We would like to thank the referee for his comments, and mention that our detailed answer to specific points is in bold below each comment.

First, regarding the beech saplings being placed in the dark for the 13C measurements following the water pulse addition. I understand the reasoning for this, to avoid respired CO2 to be assimilated, but I agree with Reviewer 1 that it may affect the results and interpretations, and there appears to be a disconnect between the physiological measurements taken under light conditions and the isotope measurements taken under dark conditions.

We agree that our rationale for leaf gas exchange measurements was probably not clear enough. We have now clarified in the text that the plants were only illuminated to assess their metabolic state, right before and after the pulse. Thus, leaf gas exchange values are used only as proxies for the plants' and mesocosms' carbon balance, because, under constant day-to-day growth conditions as in our experiment (except for the time of the water pulse), leaf gas exchange is controlled by the plant's internal carbon balance (e.g., Goldschmidt & Huber, 1992; Paul & Foyer, 2001; McCormick *et al.*, 2009). Thus, we are using relative changes in leaf gas exchange variables over time (e.g. Fig. 2 and Fig. 3) to document the underlying physiological processes. The contrasted metabolic status at pre- and post-pulse times appear clearly in the stomatal conductance, assimilation and transpiration rates, showing that the metabolism of the plants is indeed altered by the water pulse (considering the +2h measurement only, see below for the +72h issue), despite keeping the plants in the dark. As a consequence, the relation between relative changes in leaf gas exchange variables and relative changes in $\delta^{13}C$ of respired CO_2 that we measured is not likely due to artefacts, especially when considering the response at +2h after the pulse. Furthermore, we would like to highlight that the plants used for leaf gas exchange measurements were also kept in the dark, under the same conditions (including rewetting) as the plants used for measuring $\delta^{13}C$ of respired CO_2. Thus, inferring a coupling between these two sets of plants is by no means "nonsense". The plants used for leaf gas exchange measurements were only exposed to light during the measurements. We now have clarified the setup description

to avoid such misunderstandings.

With no photosynthesis, allocation of new assimilate supply is probably slowed/stopped. The effects of this are probably different for substrate use by above and below ground plant components, and particularly measurements that span from 2 to 72 hours after the water pulse, there could be large differences in C sources being used.

The referee is perfectly right when they mentioned that a plant kept in the dark will suffer from carbohydrate starvation at some point and that there might be a gradual shift in the respiration substrate as well as an impairment of phloem transport. However, such processes take time (e.g., Tcherkez et al. 2003: in French beans, starch, sucrose and glucose concentrations remained above 50% of their initial values for one to two days in the dark at 20°C. The concentrations decreases were even slower at lower temperature). Thus, as stated by the referee, measurements at +2h and +72h should be considered differently: while carbohydrate starvation is likely at +72h, it is unlikely at +2h. Measurements taken at +2h (i.e. 2 hours after the beginning of the pulse, thus after 1h45 in the dark) should not be affected significantly by the lack of new carbon, and thus can be discussed in the light of plant physiological response to the water pulse. Following the referee's comments, we now include these points in the discussion and removed all references to relation between leaf gas exchange measurements and $\delta^{13}C$ of respired CO_2 at +72h.

Nonetheless, we would like to still include the $\delta^{13}C$ of respired CO_2 data over the entire duration of the experiment, since it shows that no major changes took place after the first response to the water pulse. Indeed, several processes might be involved in the belowground response of $\delta^{13}C$ of soil CO_2 efflux to the pulse: a microbial response to rewetting (e.g. Unger et al. 2010) and C transfer from aboveground to roots and rhizopheric microbes in ungirdled trees. The timing of these processes was not known before starting the measurements. Therefore, the measurements had to be made over a period of time sufficient to ensure that none of these responses would be missed. Furthermore, $\delta^{13}C$ of soil CO_2 efflux can show some periodicity (e.g., Unger et al. 2010) and the experiment had to be performed long enough to determine whether such periodicity could be observed.

This directly impacts the conclusions, and these treatment affects need be better reconciled in the methods, discussion and conclusions.

We have modified the manuscript according to the comments of the referee (see above for specific points), and we have reframed the hypotheses accordingly: 1) the water pulse ending the drought period should alter the plant and soil metabolisms; 2) Plant metabolic changes should lead to changes in $\delta^{13}C_{R\text{-}above}$; 3) water pulse-induced changes in $\delta^{13}C_{R\text{-}soil}$ are expected to be partially driven by changes in plant metabolism, but also by changes in microbial metabolism.

Additionally, we have altered the structure of the discussion to match these hypotheses. Our discussion now includes i) a first part on the response of plant and soil metabolisms, supported by Table 1 and the updated Fig. 1 (see below), ii) a second part on $\delta^{13}C_{R\text{-}above}$ (Fig. 1D) and plant metabolism, including the discussion on changes in plant carbon balance (supported by Fig. 3A and 2D), carbon starvation and soluble organic compounds used for osmotic adjustment, iii) a third part on changes in $\delta^{13}C_{R\text{-}soil}$ (Fig. 1F) with two subsections: one discussing the contribution of changes in plant metabolism (supported by Fig. 2B), and a second subsection discussing the contribution of changes in microbial metabolism (supported by Fig. 2C).

Second, the manuscript does not show the measured CO2 flux response in a figure. It is coarsely shown in Table 3, but it would really help the reader to see the pulses of respiration from the different components over time. This should be added, and would make the manuscript more quantitative.

We agree with the referee that providing more precise information on CO_2 fluxes is important. Thus, we propose to add three panels to figure 1 to present the CO_2 efflux data (see updated Figure 1 below).

For example, does the isoflux of from above and belowground match the mesocosm isoflux?

The above- and belowground isofluxes necessarily match the mesocosm isoflux, because the aboveground isoflux was calculated from the mesocosm and belowground isofluxes (see equation 4 in the manuscript).

The authors should be able to quantify the contribution of autotrophic and heterotrophic respiration to soil respiration over time using the girdled and ungirdled treatments.

We do not think that it is possible to separate autotrophic and heterotrophic respiration, even with girdled and ungirdled trees, due to the consequences that girdling also has on exudation and therefore on rhizopheric activity. Girdling might also modify heterotrophic respiration by increasing the amount of dead roots available for decomposition. Thus, we think that the difference in soil CO_2 efflux between girdled and ungirdled pots reflects the importance of fresh carbon supply in the respiration rather than the difference between total respiration (heterotrophic and autotrophic) and heterotrophic respiration.

How does the CO2 flux from microbes that receive fresh plant inputs differ from that of microbes with no root exudates?

As shown in the updated Figure 1 (see below, panel C), soil CO_2 efflux was higher in girdled trees than in ungirdled trees, suggesting that the decomposition of roots in girdled trees provided more substrate for microbial respiration than the carbon transported from aboveground in ungirdled trees. This is an interesting point which might, however, be due to the short time plants were given for assimilation during the water pulse.

How is the CO2 flux/and isoflux timing different with plants and without plants?

No difference in the timing of the response was observed between girdled and ungirdled trees.

We agree with referee#2 that including the three points above in the results and discussion brings a better understanding of our mesocosms responses to the water pulse and thank him for these constructive comments.

Third, in the results it states that the SWC in the pots were maintained at 80% field capacity. However, in Table 1 - the girdled plot have much lower SWC than the ungirdled. This is also a treatment affect that needs to be addressed particularly for the microbial drought response.

We have improved the text to better explain that SWC was kept at 80% of field capacity during the 5 months period prior to drought, whereas the SWC given in Table 1 are the values at the end of the drought period (-24h) and after the water pulse (+1h and +72h). Differences between girdled and ungirdled mesocosms appeared during the drought period (before all were kept at 80%), but the cause of this difference remains

unclear to us.

Figure 1 should be improved to allow the reader to see the individual treatments. I suggest panels that share a y-axis instead of the x-axis, and this would be more intuitive anyway, because it is the size of the 13C shift that is highlighted not the temporal differences between the components.

Figure 1 has been changed according to referee's suggestion (see below). CO_2 fluxes were also added (see above).

References

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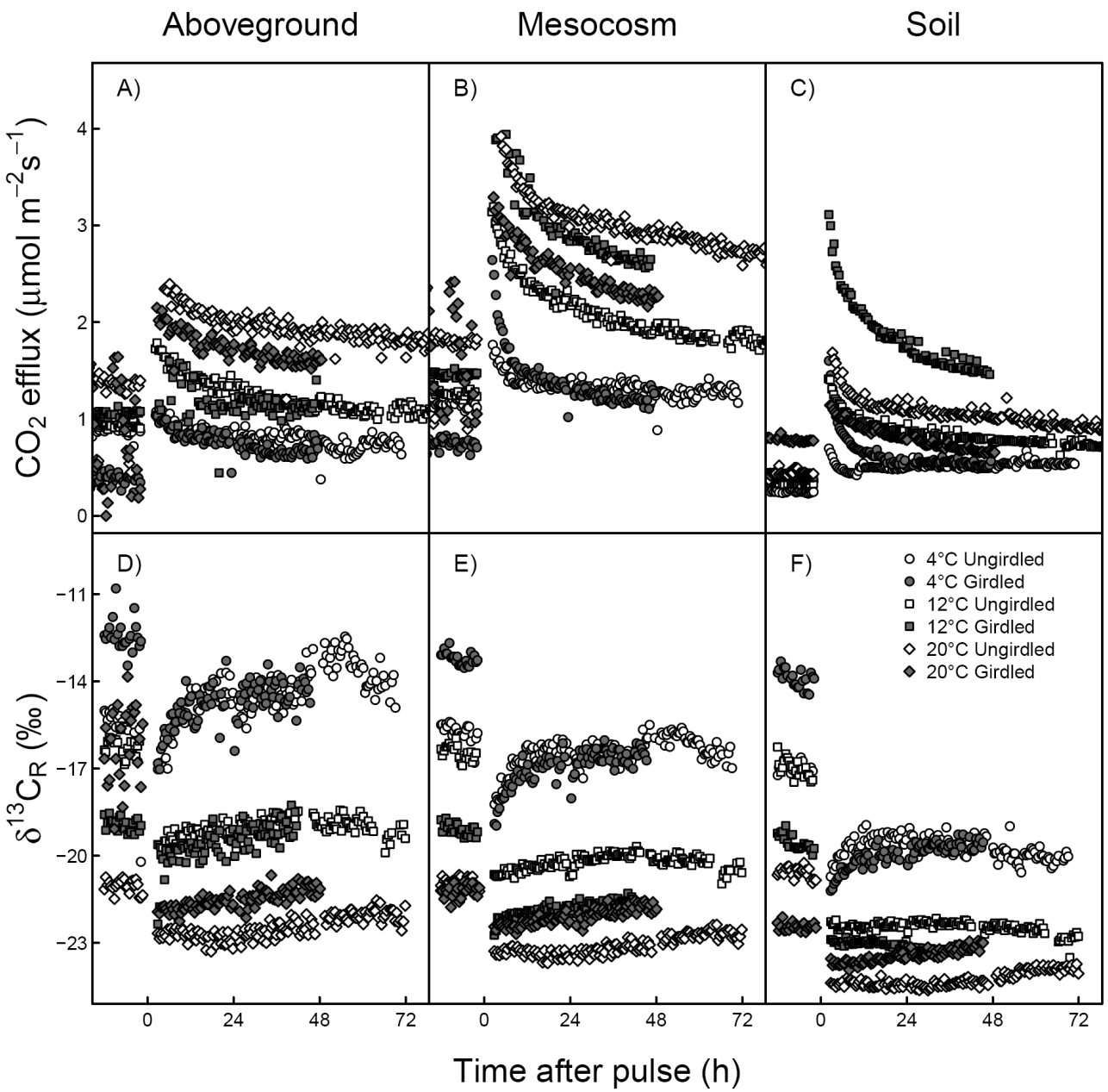
Figure 1: CO_2 efflux rate in the aboveground (F_{above}, A), mesocosm ($F_{mesocosm}$, B), and soil (F_{soil}, C) compartments, as well as $\delta^{13}C$ of aboveground respiration ($\delta^{13}C_{R\text{-}above}$, D), mesocosm respiration ($\delta^{13}C_{R\text{-}mesocosm}$, E) and soil CO_2 efflux ($\delta^{13}C_{R\text{-}soil}$, F) for beech mesocosms before and after a water pulse given at time=0. The *Fagus sylvatica* mesocosms were grown under different temperatures (4, 12 and 20°C), combined (n=1) with two girdling treatments (ungirdled and girdled). On-line IRMS measurements were performed in the dark, however, plants were exposed to light for 15min starting at the water pulse (time=0) to assimilate C immediately after the pulse.


Aboveground
Mesocosm
Soil
A)
B)
C)
D)
E)
F)
CO_2 efflux (µmol $m^{-2}s^{-1}$)
$\delta^{13}C_R$ (‰)
Time after pulse (h)
4°C Ungirdled
4°C Girdled
12°C Ungirdled
12°C Girdled
20°C Ungirdled
20°C Girdled