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

Response to referee #1:

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.

The fact that the plants were maintained in the dark after the water pulse (P500, L1-5) has stopped photosynthesis and therefore any link between the expected change in DELTA_I and delta_13C of respiration. In such conditions, hypothesis 2 but also 1 and 3 cannot be anymore tested because we expect that the response time of stomata to the water pulse was higher than the 15 minutes of photosynthesis that was allowed during the water pulse (P507, L3-10). The comparison of DELTA_I measured by leaf gas exchange of illuminated leaves on the second set of plants and the delta_13C of respiration of the first set of darkened plants (P508, L1-7) seems to be nonsense to infer a coupling between both. This is a major drawback.

We understand the referee's major concerns about the short duration of the light period that was associated with the water pulse, as well as about linking leaf gas exchange measurements carried out in the light to physiological activities in the dark (to measure $\delta^{13}C$ of respired CO_2). We agree that our rationale was probably not explained well enough and have remedied to this in our revision. Actually, the water pulse had two objectives: i) to create recent photoassimilates with a different signature that could subsequently be tracked through the plant-soil system, and ii) to alter plant and soil metabolisms and to investigate the subsequent changes in respired isotopic signatures of plant and soil.

Regarding the first objective of the water pulse (i.e., creating photosynthates with different isotopic signatures) there are two main approaches to change the isotopic signature of photosynthates. The first is to provide isotopically labelled CO_2 substrate to the plant, and the second is to abruptly change environmental conditions, and consequently change photosyntethic discrimination (Δ_i). Both of these methods result in a spike in the signature of the recently assimilated C. In a previous experiment, we found that a 15-minute ^{13}C-CO_2 pulse-chase labelling of wheat plants that were previously in the dark and that were put back in the dark after labelling was long enough for plants to synthesise enough carbohydrates with a sufficiently different isotopic signal for us to measure this signal and to track it to soil respiration during more than 48h. These data are yet unpublished, but the excess ^{13}C in soil-respired CO_2 increased within 2 hours and was still positive (10.4±4.0 $\mu g\ m^{-2}\ h^{-1}$) 48h after the

pulse. In the experiment described here, pre-experiment tests showed responses of Δ_i (calculated from measured c_i/c_a) within these 15 minutes, although the smaller isotopic difference obtained by this "natural" labelling (due to changes in water status) may have limited its magnitude. Furthermore, it is important to keep in mind that stomatal regulation is not only driven by changes in soil water content and the transport of the newly available water to the leaves, but also affected by changes in atmospheric humidity and can respond within minutes to changes in environmental conditions (Lambers et al. 1998, p185). In particular, increase of stomatal aperture in response to increase in air humidity happens in a few minutes and can be observed in the absence of leaf water content change (Lösch & Tenhunen, 1981, pp137-139 and references therein). Thus, we are confident that changes in stomatal conductance, and consequently in Δ_i, occurred during the 15 minutes of photosynthesis following the water pulse.

Regarding the second objective of the water pulse, i.e. creating plants with different physiological performance, 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.

The discussion starting from P510, L18 to P511, L26 completely hides the fact that the measurements of delta_13C were done in the dark and that no new photoassimilate were produced after pulse labelling that would have been transferred by the phloem and used as substrate for respiration. Same for P512, L2 to P513, L3: no new photoassimilate could have been transferred to the root and used as substrate for root or rhizosphere respiration via exudation.

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, 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.

Owing that, the results are surprising but amazing. The change in delta_13C of soil respiration might have been driven by change in carbon source of soil microbes (but with possible interaction between rewetting and photosynthate starvation after several hours or days in the dark).

Changes of carbon sources used for respiration by microbes are being discussed. Please, see also below, our answer to the comment "P514, L19 - P515, L8".

The change in delta_13C (respiration, phloem or microbial biomass) cannot be ascribed to change in DELTA_I (or stomatal conductance) because photosynthesis doesn't occur in the dark. It can be due to change in carbon sources related to photosynthate starvation.

Please see our response to changes of leaf gas exchange measurements and carbohydrate starvation above.

Among the new sources, you may expect the use of soluble organic compounds that were previously use for osmotic adjustement before drought was relieved by the pulse watering (can it explain the drop in delta_13C of leaf biomass?).

The referee proposes constructive additional hypotheses that we have readily included in the manuscript to improve the explanation of the measured changes in $\delta^{13}C$. In particular, we agree that soluble organic compounds used for osmotic adjustment before the pulse could play a role in the observed response of $\delta^{13}C$ of respired CO_2 measured after the pulse. Although the nature and contribution of such soluble organic compounds remains speculative in our study, it is likely that these compounds were synthesised from carbon assimilated during the drought period and that they became more abundant as drought stress increased. A gradual increase of drought intensity also leads to a decrease in Δ_i. Therefore, such compounds accumulated during the whole drought period should have an isotopic signature which also integrate Δ_i over the whole drought period and thus have a lower $\delta^{13}C$ values than carbon assimilated at the end of the drought, just before being placed in the dark. Hence, their respiration could lead to a decrease in $\delta^{13}C$ of respired CO_2 after the water pulse.

The significant relationships found between gs (orCi/Ca) and delta_13C in respiration, phloem or microbial biomass may be more likely due to some confounding factors.

We agree that changes in $\delta^{13}C$ in relation to changes in g_s are likely not driven by g_s itself, but probably results from changes in C sink strength which would impact both g_s and $\delta^{13}C$. Such a link between g_s and carbon sink strength has been observed before (e.g., Koller & Thorne, 1978; Peet & Kramer, 1980; Goldschmidt & Huber, 1992) and therefore seems plausible in our experiment.

The manuscript should probably be rewritten to explore these putative explanations (and other), but without any attempt to relate what measured on dark adapted plants and on illuminated leaves.

Points discussed above in the answers to the referees' comments on both duration of the pulse and relation between leaf gas exchange measurements in the light and $\delta^{13}C$ of respired CO_2 measurements for plants kept in the dark have been added to the discussion.

Furthermore, we have reframed the hypotheses accordingly: 1) the water pulse after the drought period should alter the metabolism of both plant and soil; 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).

Additional points P497, L20: The root system of 1m tall beech sapling may extent well above 9 cm of the stem and below 17 cm depth. Can you provide indication about the severity of root disturbance induced by collecting the trees? This is a quite big issue for understanding the response of the tree to imposed drought. One option will be to give the sapling density in the original forest and the rooting depth of the sapling so that the average soil volume available for each sapling in natura can be estimated.

We agree with the referee that part of the rooting system might have been severed during the tree collection, including damage to fine roots which is difficult to assess. Unfortunately, data about rooting depth and density are not available. However, when setting up the experiment, we collected more beech saplings than needed and selected for the experiment only those individuals with no major visible damage of the root system, in particular those presenting no cut coarse roots. Furthermore, during the five months preceding the drought, all the saplings grew enough fine roots to colonize the entire pots, therefore all saplings used during this experiment had a functional and similar rooting system (personal observation at the end of the experiment when the soil and root materials were sampled). We now explain that in the method section.

P514, L19 - P515, L8: this part of the discussion is an interesting review but quite speculative to interpret the data without additional measurements like isotope composition of specific organic compounds.

We agree with the referee that this part of the discussion is quite speculative in the absence of compound-specific measurements in the soil, and that we cannot determine which organic carbon source is actually respired. Nonetheless, we think that our results can provide some hints about the carbon source that might be of interest for the readers. We now state more clearly the speculative nature of this paragraph.

References

Goldschmidt, E. E., Huber, S. C.: Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars, Plant Physiology, 99, 1443-1448, 1992.

Koller, H. R., Thorne, J. H.: Soybean pod removal alters leaf diffusion resistance and leaflet orientation, Crop Science, 18, 305-307, 1978.

Lambers, H., Chapin, F. S., Pons, T.: Plant physiological ecology, Plant physiological ecology, New York, USA, Springer 17. 1998.

Lösch, R., Tenhunen, J. D.: Stomatal responses to humidity - phenomenon and mechanism. Stomata Physiology - (Society for Experimental Biology. Seminar series; 8), P. G. Jarvis and T. A. Mansfield, Cambridge, UK, Cambridge University Press, 1981.

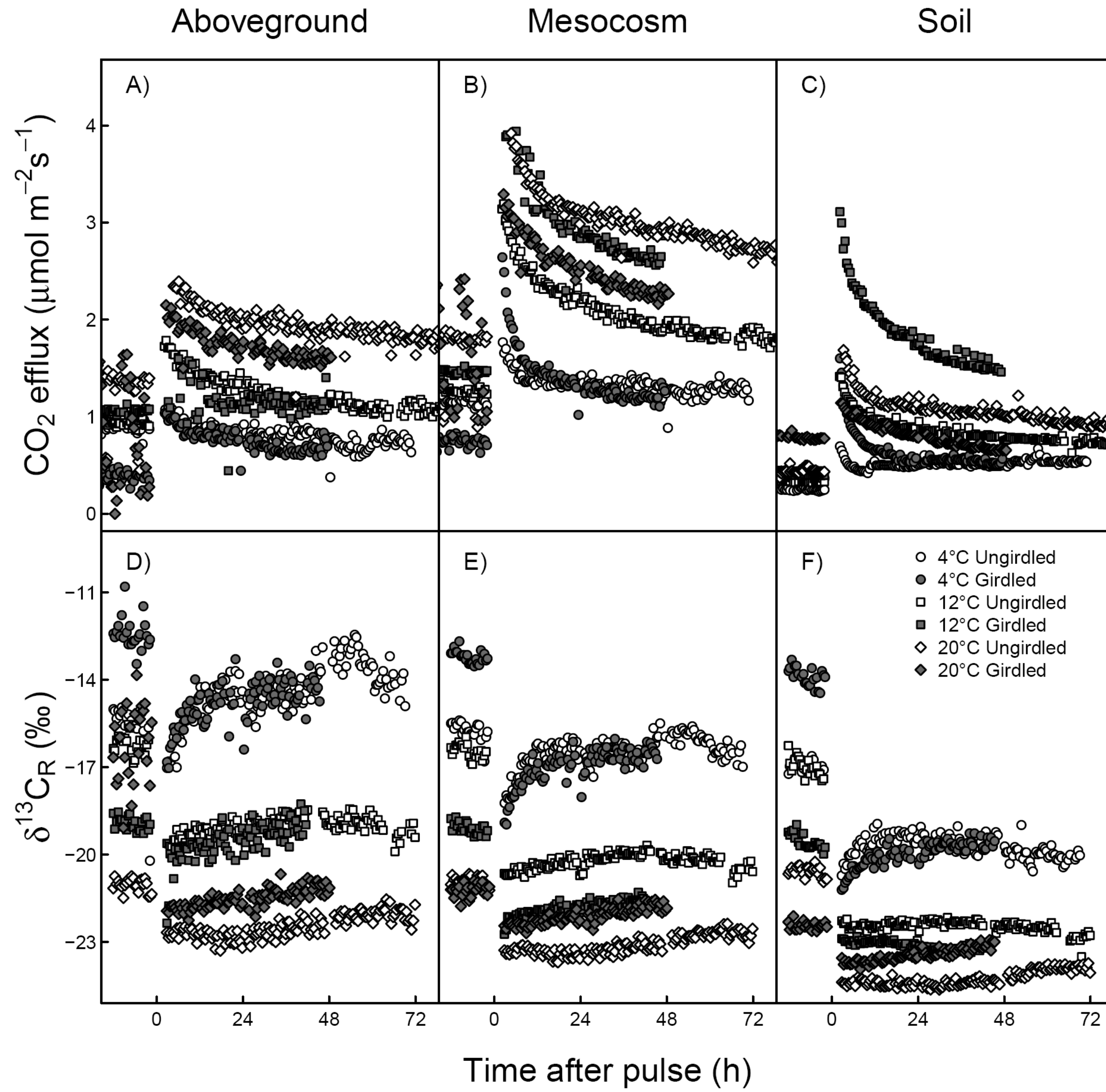
McCormick, A. J., Watt, D. A., Cramer, M.D.: Supply and demand: sink regulation of sugar accumulation in sugarcane, Journal of Experimental Botany, 60, 357-364, 2009.

Paul, M. J., Foyer, C. H.: Sink regulation of photosynthesis, Journal of Experimental Botany, 52, 1383-1400, 2001.

Tcherkez, G., S. Nogués, Bleton, J., Cornic, G., Badeck, F., Ghashghaie, J.: Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in french bean, Plant Physiology, 131, 237-244, 2003.

Unger, S., Máguas, C., Pereira, J. S., David, T. S., and Werner, C.: The influence of precipitation pulses on soil respiration - Assessing the “Birch effect” by stable carbon isotopes, Soil Biology and Biochemistry, 42, 1800-1810, 2010b.

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})
$\delta^{13}C_R$ (‰)
4°C Ungirdled
4°C Girdled
12°C Ungirdled
12°C Girdled
20°C Ungirdled
20°C Girdled
Time after pulse (h)