

General comments Referee #1

Does the paper contain enough new and relevant data that will make a research paper rather than a technical note? I guess the authors have thought 'no' as soon as they as submitted it as a technical note. The tight coupling has been described several times and the fact that the experiment is done in the lab on small seedlings is less existing than similar experience done in the field. The same apply for the suggestion that diurnal variation in ^{13}C composition of respired CO_2 can be explain by alternative use of starch/new photosynthate during day night cycles (Bahn et al. 2009, Plain et al 2009)

Answer: Performing a ^{13}C canopy pulse labeling in order to study atmosphere-plant-soil interactions was a straight forward case study in order to test our chamber setup for the suitability of continuous measurements with weeks of duration. Similar chamber systems reported elsewhere, especially for experiments on grasses, were little proof for us that this approach would work with tree saplings and laser spectroscopy for measurements over days to weeks. Therefore, we still needed to test this new setup before we turn to more detailed experiments within this research field.

Additional comments Referee #1

Why are you using the los gatos equipment? You claim that this instrument is capable for in-situ measurements of the water vapour isotopic composition in air ($^{18}\text{O}/^{16}\text{O}$; $^2\text{H}/^1\text{H}$) and its respective mixing ratios, but no results are shown.

Answer: The Los Gatos Laser was used to measure water vapour mixing ratios in order to quantify transpiration and conductance. The water vapour isotope data is not indented to be published in this study.

General comments Referee #2

Regarding the experiment itself, two of 6 beech saplings were excluded from the study on the basis that they were obtained from a different tree nursery, and had been planted in different soil. This considerably reduces the value of the study, as no statistically sound comparison between the droughted and watered saplings can be made, and the results can only be regarded as "proof of concept". The fact that the authors have submitted this as a "Technical Note" probably reflects this realisation. To that end, the exact origin of the saplings and the soil type should not matter, as the objective is to show the potential of the novel chamber design for labelling, with no direct contamination of the soil compartment by atmospheric label diffusion.

Answer: The aim of our manuscript was to show and discuss the application of this chamber system coupled with a laser spectrometer for potted beech saplings and not to focus on a drought versus control experiment. We, nevertheless, decided to exclude the two trees from the different nursery to have a consistent dataset. We are aware that the exclusion of two trees reduced the value of this study and have ensured an identical soil and tree origin in consecutive experiments.

Somewhat more worrying is the fact that in one of the remaining four pots (and seemingly also in one of the excluded ones) the critical seal between canopy and soil compartment was not adequate, resulting in contamination. If this system has a “failure” rate of 1 in 3, it will be of little use. The authors should indicate the likely source of the problem, and whether this is an inherent risk of the set-up, or if it can be avoided in the future.

Answer: There was a failure rate of 1 in 6. The contaminated sample was “coincidentally” one which has been excluded from the result section. The biggest challenge when applying such a complex technical setup is in achieving a complete gas-tight seal between compartments. We showed the contaminated sample in order to discuss the effects of contamination in such a setup. The gained experience concerning contamination and leaks is helpful to prevent similar errors in future experiments.

Detailed comments Referee #2

1604, 8/9: Remove manufacturer’s names in the abstract.

Answer: We have made the suggested changes.

1605, 12: 50% is a fairly approximate number, and the actual range is wider, dependent on ecosystem season, etc. I would prefer to see reviews or meta analyses cited to make this general point, as there are a large number of studies on this and it isn’t clear why you choose to use these particular ones.

Answer: We have added the reference.

1605, 13: “plant’s” or “plants”’, rather than “plants”

Answer: We have made the suggested changes.

1605, 13: A citation is needed for the assertion regarding the plant carbon budget.

Answer: We have added the reference.

1605, 29: “determining” is not true, I suggest substituting with “influencing”. Delete “even”.

Answer: We have made the suggested changes.

1607, 17-19: Delete sentence. This specific detail is explained later, and is not relevant to the experimental design described in this paragraph.

Answer: We have made the suggested changes.

1608, 16: “flow-through” rather than “through-flow” (?).

Answer: We have made the suggested changes.

1608, 20: Please clarify if the soil compartment was sealed against the wooden boards, or if these were structural parts only, with no gas seals formed between wood and PVC or Plexiglas. Wood is not a good material if you want to achieve gas tightness, and if wet, can be a source of CO₂.

Answer: Yes, the soil compartment was sealed with rubber gasket against the wooden boards. The wooden boards are varnished, so that even they were getting wet, it should not be a source of CO₂.

1608, 21: Delete “Because of the canopy”.

Answer: We have made the suggested changes.

1608, 22: Did you seal the joint between the two semi-circular discs?

Answer: Indeed, the joint was sealed with Terostat also.

1609, 5-9: Move these sentences to the Results section

Answer: We have made the suggested changes.

1609, 10/11: I don’t think the exact model of fan is necessary to include. Also: 16.5 l/min seems an extremely high flowrate.

Answer: We have made the suggested changes. We selected a fan with a high flow rate in order to reduce boundary layer effects and to mix the air well inside the chambers. The flow rate of 16.5 L/s is given by the manufacturer.

1609, 11: Replace “a well” with “good”

Answer: We have made the suggested changes.

1609, 24: State manufacturers of laser spectrometers here.

Answer: We have made the suggested changes.

1610, 21: What is SERTOflex? Tubing?

Yes, SERTOflex is the tubing which was used. We changed it to “tubing (SERTOflex)”.

1610, 26: Delete “chemically”.

Answer: We have made the suggested changes.

1611, 1: Not strictly true; what do you suppose happened to the sulphate and the sodium?

Answer: Yes, that’s right. Sodium sulphate is another endproduct of this reaction.

1611, 5: This is new to me. From own experience, I know that our Li-Cor gas analysers (admittedly a different model) “misses” about 2/3 of all ^{13}C . Did you test the ^{13}C sensitivity of this analyser yourself? I would be surprised if this particular model was more sensitive to the isotope than others by Li-Cor.

Answer: We tested the Li-cor analysers again. The 6262 model misses about 80% of all ^{13}C . New values were re-calculated and implemented into the manuscript.

1611, 21: Delete “commercially available”

Answer: We have made the suggested changes.

1612, 17: As $d^{13}\text{C}$ is always expressed as per mille, please include “x 1000” in your equation for completeness.

Answer: We have made the suggested changes.

1613, 14: “replicates” rather than “samples”.

Answer: We have made the suggested changes.

1614, 14: Better expressed in hours than minutes.

Answer: We have made the suggested changes.

1614, 17-18: This is quite speculative (which is ok), but physiologically not very plausible. Phloem transport is diffusive, rather than a mass flow (mainly), and xylem pressure should not influence it by much. (In fact, high transpiration should lower xylem pres-

sure, as this is how water moves in the stem.) Unless you have evidence from literature that these correlate, I think that your data are only very poor proof for such a mechanism.

Answer: We agree on the fact, that transpiration might not be a direct driver of phloem transport rates for the named reasons stated by you. Alternatively, photosynthesis seems more likely to drive these patterns, as more sugars will be loaded to the phloem, hence increase transport velocities. We will address this issue in consecutive experiments and implement the respective changes in the manuscript, namely by removing the statement that transpiration might be an indirect driver of phloem transport velocities.

1614, 22/23: You never describe how you measure conductance or transpiration, but it is necessary to understand this here. If transpiration correlates with phloem transport speed, but photosynthesis doesn't, does this mean that water use efficiencies differed between replicates?

Answer: We calculated conductance and transpiration from the measured water vapour mixing ratios obtained from the Los Gatos laser spectrometer. We agree that the number of replicates is supposedly too small to draw firm conclusions about the correlation between transpiration and phloem transport rates and about water use efficiency. See also previous answer.

1615, 4/5: I can't see any evidence for photosynthesis driving the diurnal 13C pattern. The phase of the fluctuation not 24 hours, but the light cycle is, and the insert in Fig 5 does not illustrate very well what you describe here.

Answer: The suggested starch theory does only apply for the first 24 h after labeling. We strongly consider that the second occurring peak in 13C soil respiration is due to starch release which has been accumulated within the preceding light period. During the first night after labelling it can be clearly seen that there is a second, strong label release which is most likely a discharge from storage pools. Such a relationship between the 13C diurnal cycle and starch accumulation/release has also been previously found by Gessler et al. (2007) and Brandes et al. (2006) for twig phloem exudates. Also Bahn et al. (2009) suggest that the observed diurnal cycles in their conducted study relates to a starch metabolism.

1615, 10-17: don't quite follow how the starch/remobilisation issue is relevant here. Transitory starch reserves in leaf chloroplasts will certainly be labelled, but if they were activated at night time, it would take about 20 hours for them to be visible in soil respiration. If the Tcherkez et al model relates to sucrose being enriched over natural abundance levels of bulk starch, your enrichments are several orders of magnitude greater than that, so that this effect is not relevant. The Gessler and Koduma study

likewise.

Answer: It has been shown by Zeeman et al., (2007) that in *A. thaliana* starch degradation from leaf chloroplasts is initiated immediately after sunset and subsequently transported to various sink tissues within the plant. Again, we repeat what is written in the manuscript already “Transitory starch, synthesized in chloroplasts during the day, provides a steady and sufficient supply of carbon for growth, sucrose synthesis and respiration throughout the subsequent night (Zeeman et al., 2007)”

We further agree on the Tcherkez issue and implemented the respective change in the manuscript.

1615, 28-1616, 6: There may be a more consistent pattern if you calculated label-derived $^{13}\text{C}\text{CO}_2$ soil efflux. A higher ^{13}C abundance in non-watered samples appears to be coupled with a reduced soil CO_2 efflux rate, so that the total amount of label returned this way may be similar?

Answer: We attached a graph (Fig 1) showing the difference between in- and outlet of the soil chamber as a direct measure of ^{13}C label released by the soil. That figure shows, as suggested by the reviewer, that differences among treatments are smaller. If we do not account for the respective soil respiration rates, strong daily cycles do occur due to the different strength of soil respiration. Overall it is difficult to account for the total $^{13}\text{C}\text{CO}_2$ flux, since different soil watering regimes affect the contribution of heterotrophic respiration to total respiration.

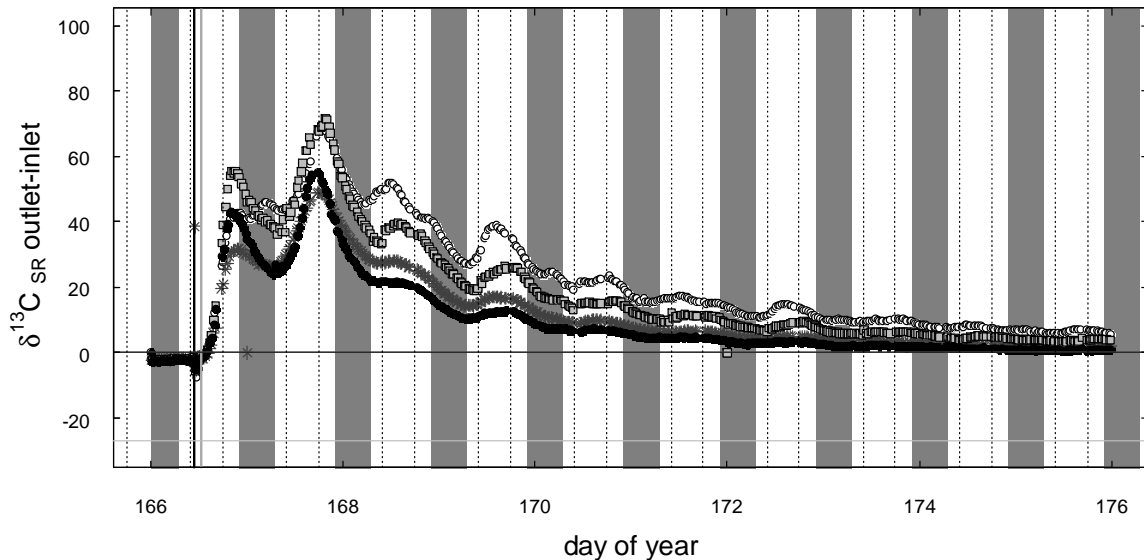


Fig.1: Symbols are the same as used in the manuscript. Open symbols denote non-watered, closed symbols well watered replicates.

1618, 14-17: I don't think you can conclude starch dynamics on the basis of your results (see above), and this speculation is not required in the conclusion.

Answer: We have made the suggested changes.

Figure 1: "iso = isolation"? Do you mean "seal"?

Answer: Yes, "iso" is supposed to mean seal. We will made the suggest changes.

Figure 4: What are the dotted vertical lines? It would be interesting to know soil temperature also.

References

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