

Hess-2020-404 Author reply on comment from Anonymous Referee #2:

We thank Reviewer #2 for the constructive feedback. Please find below our responses to the individual comments and suggestions (reviewer #2 comments in blue font, with our response in black font).

The main objective of this study was to investigate plant water sources.

First, I should say that after the work of Brooks et al. (2020), countless studies across regions (including tropical wet environments; see Goldsmith et al. 2012; Muñoz-Villers et al. 2018) and revisions have showed that plants use evaporatively fractionated soil water (Sprenger et al., 2016; Sprenger and Allen 2020). The general finding is that plant water is isotopically similar to bulk soil water but not to low suction lysimeter water, implying that roots are generally located in less conductive (mobile) pores where water tends to travel more slowly and can reside for longer times.

We agree with Reviewer 2 and recognize the important work of the Brooks et al. 2010 and others which were also cited in our manuscript. Seeing the on-going discussion around which water plants use (Berry et al., 2018; Sprenger and Allen, 2020) and as presented in our manuscript that the majority of the plant water studies focused on trees in natural catchments. Instead, as stated in L110-113: isotopes have been used to a lesser extent in agricultural systems than in natural systems to investigate plant water sources (Penna et al., 2020). There are successful studies done in coffee (Muñoz-Villers et al., 2020), maize, wheat (Stumpp et al., 2009) and rice cultures (Mahindawansa et al., 2018; Shen et al., 2015). This shows the need to continue and explore the potential of stable isotopes of the water in ecohydrological studies, especially in agricultural settings and novel aspect of this study.

As described in L103 the soil water can consist of different pools of water: "...plants use mobile vs. immobile soil water pools (Brooks et al., 2010)...". To infer which water plants use, lysimeters are commonly used to sample mobile soil water (Sprenger et al., 2015). Instead to access the tighter bound and more fractionated soil water with matric potential < -0.1 MPa, the immobile soil water, it is necessary to use e.g. the cryogenic vacuum method (Sprenger et al., 2015). Therefore, we agree with the reviewer that all soil water collected with lysimeters should be considered mobile soil water.

To highlight and avoid confusion we distinguish between mobile and immobile water in the new version of the manuscript.

In the present study, soil water samples representing the soil water pool for plants, were only collected using low suction (80 kPa) lysimeters. Bulk soil samples were a key part of the experiment but they are missing here. This methodological issue, is perhaps the largest flaws of the research and I do not see a way to get out of it, based on all the evidence published over the last decade.

In addition, your soils are dominated by clay content (Table A1) which is very well known for its very fine particle structure making very difficult to empty the water from such smaller pores using low soil tension lysimeters. This is other reason why the authors should have collected and used bulk soil water isotope ratios (from cryogenic vacuum distillation) as the representative soil water source for plants.

We agree with the reviewer that soil water collected with lysimeters should be considered mobile water (consistent with the comment from Reviewer #1). To have information on the full spectrum of the mobile and immobile soil water we did collect soil cores for bulk soil water extraction, especially in low

matric potential situations as in Period III. On average, however, we could extract less than 0.1 ml of water per soil sample using cryogenic vacuum extraction. This was too little water for pipetting and analysis in the LIS-autosampler setup. As a result, we could not gain information on the immobile water isotopic composition. As a result, we could not gain information on the bulk isotopic composition. Initially we decided to not include this information in the manuscript but feel to it is necessary include this in the method section of our manuscript and

After Old L224:

In addition to the lysimeters, soil samples for bulk soil water extraction and subsequent stable isotope analysis in were collected randomly in all plots from a depth of ~10 cm on 7 out of 11 sampling days. In order to not disturb the rice plants, instead of an auger, a steel rod 50 cm in length with a 2cm diameter was pushed 10 cm into the soil. After removing 5 cm of the topsoil, the soil sample was collected (~5 cm Ø2cm). The sample was then placed in a double re-sealable zipper storage bag. To minimize post-sampling evaporation, the storage bags were directly placed in a cooler with ice. All soil samples were stored in the laboratory freezer (-80 °C) before extracting the soil water for isotopic analysis.

And old L235-246:

Plant water was extracted from the stem (xylem water) of the different rice plants to infer which sources of water the rice plants used. We used the cryogenic vacuum extraction technique described by Koeniger et al., (2011) to extract the plant and bulk soil water for stable isotope analysis. The method uses a heated vial and a cold trap vial (Exetainer® vial with standard cap and rubber septum, Labco Ltd, Lampeter, United Kingdom) connected with stainless-steel capillary tubing. About 3 g of plant material from the rice stem was placed in the heated vial before the system was evacuated to 85 kPa with a vacuum hand pump (Mityvac). The heated vial was heated for 1 hour at 100°C using a test tube heater (HI839800 COD Test Tube Heater; Hanna instruments) while the cold trap vial rested in a Dewar flask containing liquid nitrogen at about -196°C. After the extraction was stopped, the cold trap vial was sealed with Parafilm and left to thaw. After thawing, the extracted liquid water was pipetted into 2 ml vials (32 x 11.6 mm screw neck vials with cap and PTFE/silicone/PTFE septa) and stored refrigerated (5 °C) until stable isotope analysis. The plant root and bulk soil water was extracted in the same manner as the xylem water using the cryogenic vacuum extraction technique but with extraction time longer than 3 hours. On average 86±5 % plant water and soil water were extracted. However, we extracted less than 0.1 ml of water per soil sample for the bulk soil water and less than 0.1 ml of water per root sample for the root water which were too small volumes for pipetting and the LIS-autosampler setup.

We felt due to the missing bulk soil water information to not calculate the fraction of different plant water sources and over interpret our data. However, to comply with the Reviewer #1 request we quantified, based on the available isotopic data, the fraction of different plant water sources to better compare the plant water use in biochar amended treatments with the control treatment. Mixing models are powerful tools to estimate the plant water sources (Layman et al., 2012; Rothfuss and Javaux, 2017).

The end-member mixing model will be presented in the method section after L328 as:

In addition, in the different treatments the potential plant water use of the rice plants was quantified using mixing model. Mixing models are powerful tools to estimate the plant water sources (Layman et al., 2012; Rothfuss and Javaux, 2017). However, applying Bayesian mixing models would not decrease the uncertainty in potential plant water sources due to the missing bulk soil water isotope data due to the little amount of cryogenically extracted bulk soil water. Instead, a simple three end-member mixing model was used:

$$O_{PW} = f_R O_R + f_{SI} O_{SI} + f_{SM} O_{SM} \quad (9)$$

$$D_{PW} = f_R D_R + f_{SI} D_{SI} + f_{SM} D_{SM} \quad (10)$$

$$1 = f_R + f_{SI} + f_{SM} \quad (11)$$

$$f_R = \frac{O_{PW} D_{SM} - O_{PW} D_{SI} + O_{SM} D_{SI} - O_{SM} D_{PW} + O_{SI} D_{PW} - O_{SI} D_{SM}}{O_R D_{SM} - O_R D_{SI} + O_{SM} D_{SI} - O_{SM} D_R + O_{SI} O_R - O_{SI} D_{SM}} \quad (12)$$

$$f_{SI} = \frac{O_{PW} D_{SI} - O_{PW} D_R + O_R D_{PW} - O_R D_{SI} + O_{SI} D_R - O_{SI} D_{PW}}{O_R D_{SM} - O_R D_{SI} + D_{SM} D_{SI} - D_{SM} O_R + O_{SI} D_R - O_{SI} D_{SM}} \quad (13)$$

$$f_{SM} = \frac{O_{PW} D_R - O_{PW} D_{SM} + O_R D_{SM} - O_R D_{PW} + O_{SM} D_{PW} - O_{SM} D_R}{O_R D_{SM} - O_R D_{SI} + O_{SM} D_{SI} - O_{SM} D_R + O_{SI} D_R - O_{SI} D_{SM}} \quad (14)$$

where f_R, f_{SI}, f_{SM} [-] represents the fraction of plant water source of soil water from antecedent rainfall, immobile and mobile soil water while O and D indicate $\delta^{18}O$ and δ^2H [‰] respectively where subscript PW, R, SI and SM indicate the plant water, soil water with signature from antecedent rainwater, immobile and mobile soil water respectively. Seeing the limitation of the missing immobile water the three end-member mixing model was rather used as an explorative approach to support the more descriptive data analysis to explain potential plant water use of the rice plants in the different treatments.

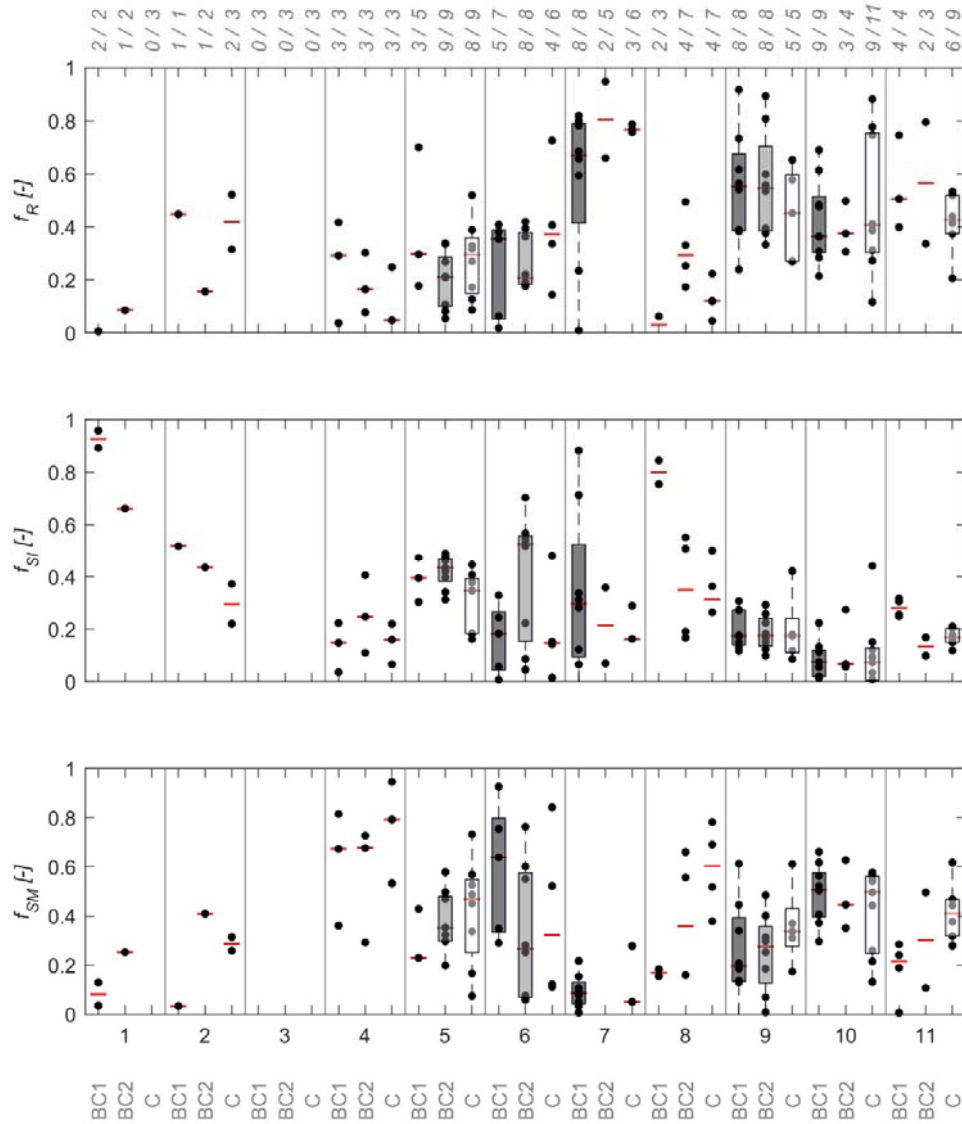


Figure 9 The fraction of plant water source rainfall (top), soil water from antecedent rainfall (middle) and the mobile soil water (bottom) indicated for each plant water sample (circle), the median fraction of the treatment (red line) and box plots ($n>5$) for the different treatments BC1, BC2 and C (indicated with grey letters) for the different sampling days 1-11. The boxes show the range of values for different sample groups (showing the median and the interquartile range, with whiskers indicating 10th and 90th percentiles). Italic grey numbers on the top panel indicate the number of times it was possible to calculate the fraction of different plant water sources out of the total of plant water samples of a treatment on a sampling day.

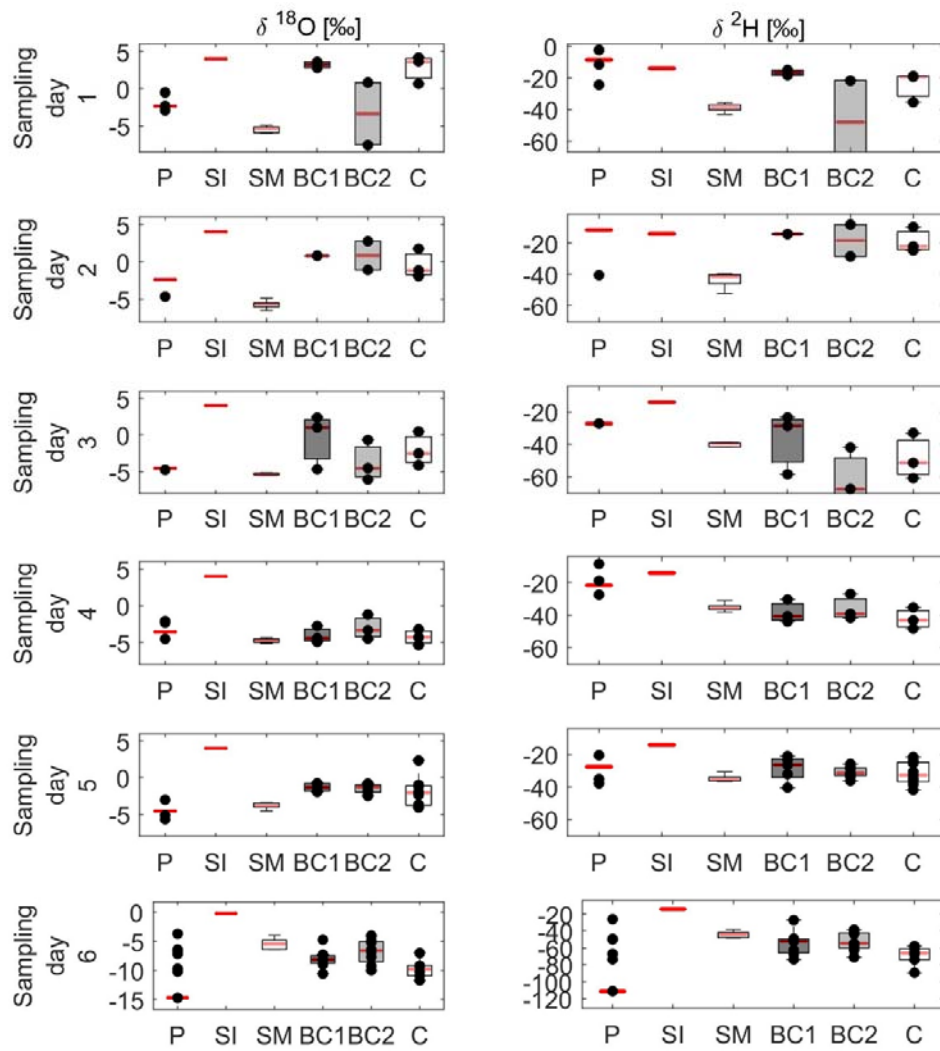


Figure A4 The stable isotope composition $\delta^{18}\text{O}$ (left) and $\delta^2\text{H}$ (right) for precipitation (P), immobile water (SI), mobile water (SM) and the plant water of the BC1, BC2 and control treatment for the sampling days 1-6 (rows). Circles indicate the data points. The boxes show the range of values for different sample groups (showing the median and the interquartile range, with whiskers indicating 10th and 90th percentiles). The red bar indicates the used end-member used in the mixing model (P-SM) and the median value for BC1, BC2 and C.

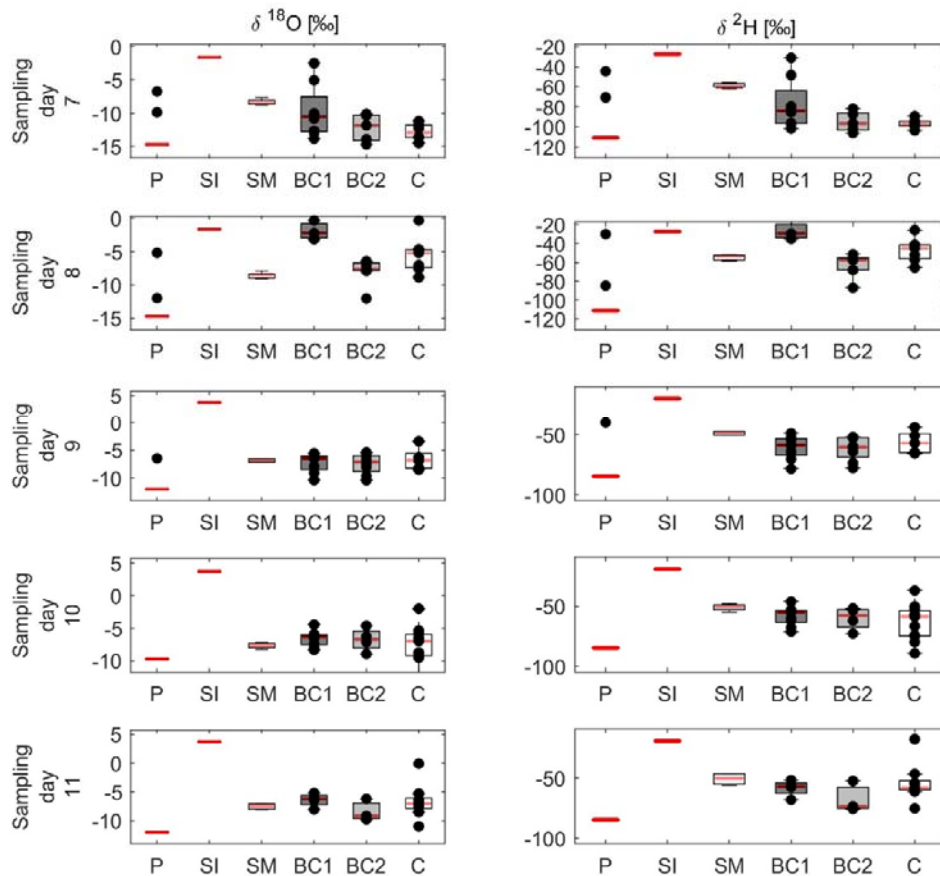


Figure A4 (continue) The stable isotope composition $\delta^{18}\text{O}$ (left) and $\delta^2\text{H}$ (right) for precipitation (P), immobile water (SI), mobile water (SM) and plant water of the BC1, BC2 and control treatment for the sampling days 7-11 (rows). Circles indicate the data points. The boxes show the range of values for different sample groups (showing the median and the interquartile range, with whiskers indicating 10th and 90th percentiles). The red bar indicates the used end-member used in the mixing model (P-SM) and the median value for BC1, BC2 and C.

The results and limitation were discussed in 5.3 Temporally variable plant water sources after L510:

The rice plants in this study had different water sources available during different periods of the experiment, but what water did they consume?

Mixing model results in Period I (Figure 9), indicated that it is likely that the plants consumed dominantly immobile. This is consistent with results observed in previous studies using stable water isotopes to map out plant water sources (Brooks et al., 2010; Penna et al., 2020; Sprenger et al., 2016). This interpretation of plant water composition is supported by plant water samples falling along the theoretical evaporation lines estimating how soil water would evolve isotopically due to evaporation (Figure 7). Therefore, it is likely that during Period I, the young rice plants (with shallow root system <20 cm as reported by Mahindawansha et al. 2018) consumed the immobile (Figure 7 and 9) which was not sampled with the lysimeters at 15 cm

and 40 cm below the surface. We could, unfortunately, not confirm this as we could not extract enough bulk soil water for isotopic analysis.

During Period II, plants grew to their maximum heights with roots reaching deeper soil layers (depth >60 cm as reported by Mahindawansa et al. 2018). This means that the rice plants, similar to larger vegetation (Allen et al., 2019), would have had access to deeper and more-stable pools of water with a distinct lower *d-excess* signature. However, the isotopic composition of plant water during this period followed the GMWL (Figure 7 b, e and h) and mixing model results (Figure 9), indicating that plants consumed largely shallow soil water from recent rainfall.

In Period III, it became increasingly difficult to extract water from lysimeters at 15 cm below the surface and the isotopic composition of plant water drifted from the GMWL, falling along the theoretical evaporation line of residual rainfall falling in Period II (Figure 7 l, o and r) which is supported by the mixing model results (Figure 9). With the experiment being held in the tropics and based on the findings from Amin et al (2020) one would expect that the rice plants with their longer roots would access the more stable and older water stores in deeper subsurface zones below 60 cm. Instead, the rice plants in the different treatments preferably consumed the temporally variable and “newer” surface near soil water from recent rainfall similarly to what has been documented in grasslands (Bachmann et al., 2015) and catchments (e.g. van der Velde et al., 2015).

As previously stated, mixing models are only as good as the available data. Despite the drawback and source of uncertainty in the simple mixing model due to the missing bulk soil water isotopic composition, the results were still useful as an explorative tool to support the more qualitative data analysis (Figure 5-8 and A4). Our results provided a first insight that plant water sources were largely variable within a treatment but no difference between biochar and control treatments could be observed. Seeing the large uncertainty and variability of plant water sources demonstrates the need of further and more detailed research of plant water use in biochar amendments. By performing more detailed isotopic experiments (Beyer et al., 2016), higher temporal resolution sampling of plant water (Marshall et al., 2020; Volkmann et al., 2016) and spatiotemporal soil water (Sprenger et al., 2015) or including interception, transpiration and atmospheric processes into the experimental analysis (Jiménez-Rodríguez et al., 2020) which would allow to not only distinguish whether the rice plants prefer mobile or immobile water (Berry et al., 2018; Brooks et al., 2010; McDonnell, 2014; Muñoz-Villers et al., 2020) but also to more accurately quantify the fraction of water sources. Next to the aforementioned vertical processes, the lateral water fluxes (Sprenger and Allen, 2020) need to be considered to assess the field-scale responses to biochar amendments (Fischer et al., 2018) which would allow to better constrain the dominant ecohydrological process as e.g. Muñoz-Villers et al. (2020). Furthermore, when mixing biochar in the top soil, a multi-layer soil profile is created and based on studies in natural catchments, e.g. Penna et al. (2018) or Sprenger et al. (2016), these different layers could store not only different quantities of water but also water characterized by different ages. In addition, Blanco-Canqui (2017) discusses how biochar can age thereby altering the physical and chemical characteristics of biochar and soil in time. The long-term effect of biochar soil amendments in tropical agroecosystems would also need

to be considered especially to understand and identify which sources of water the rice plants consume from year to year.

Although these more detailed short and long-term analyses were beyond the scope of this initial investigation our results from one growing season indicate that rice plants growing in biochar amended soils not only had access to more water (Figure 4) but also had a more stable source of green water (i.e., soil moisture from rainfall) and thus could withstand dry spells seven days longer (Figure 3). Regardless of the potential advantages, as stated by Fischer et al. (2018), it must be noted that biochar as water management tool does not adhere to a one size fits all approach but needs fine tuning in accordance with climate, site and plant characteristics to obtain stable and optimal yields.

I also observed that your samplings 9,10 and 11 corresponding to Period III (Figure 4), were characterized by low soil water contents held at very high tensions (close to PWP conditions). Hence, the soil water collected with soil lysimeters was not “seeing” the water that plants were extracted during this dry period. This situation is particularly observed for the biochar amended treatments. Therefore, the research question 2 cannot be answered.

It is correct that not all lysimeter could extract water during the dry period III. This of course does not imply that plants cannot extract water from the soil matrix as also demonstrated by different other studies e.g. (Brooks et al., 2010). We agree also with the reviewer that soil water collected with lysimeters is considered mobile water and as stated previously we will highlight this term in the manuscript.

Without overinterpreting the outcome of the mixing model, as stated, we see the use of the mixing model rather as supportive and explorative tool.

The results of the qualitative data analysis and the simple mixing model show similar results:

- 1) The isotopic composition of plant water has a large variability which is similar in all three treatments (Figure 5 and 6).
- 2) The isotopic composition of plant water has a large variability in the dual isotope space and similar temporal pattern in the different treatments (Figure 7) which can be related plant water use from soil moisture from recent rainfall, fractionated soil moisture from recent rainfall and mobile soil water.
- 3) The results of the mixing model show quantify the proportions of the aforementioned different plant water sources and show similarly a large variability.

Despite the draw back and source of uncertainty in the simple mixing model, caused by the missing bulk soil water isotopic composition, when used with care the results were a useful as explorative tool to support the more qualitative data analysis (Figure 5-8 and A4). Overall, it shows that the isotopic composition of the plant water samples across different treatments had a large within treatment variability but small difference between treatments. Which answers our research question 2) do rice plants grown in biochar amended soils access different pools of water compared to those grown in non-amended soils?

However, we also highlight that seeing the large uncertainty and variability of plant water sources shows the need of further and more detailed research of plant water use in biochar amendments.

I have made some other important comments that the authors can also consider when preparing other articles around these topics:

1) The use of mixing models to quantify the relative contributions of the different plant water sources, instead of reporting the results in a visual graphical and/or descriptive way only.

Similar to Reviewer #1, we agree and thank for Reviewer #2 pointing us that we presented and described our collected data rather qualitatively. We felt that due to the lack of bulk soil water information it would be rather uncertain and potentially erroneous to calculate the fraction of different plant water sources, which could lead us to over interpret our data. However, we agree that a better job needs to be done quantifying potential plant sources to avoid being overly qualitative. Thus, we have quantified, based on the available isotopic data, the fraction of different plant water sources to better compare the plant water use in biochar amended treatments with the control treatment.

Although we see the potential of Bayesian mixing models we agree with Layman et al. (2012) statement that “including Bayesian-based approaches, are not a quick fix or a substitute for poor sampling strategy”. Or as in our case, applying Bayesian mixing models would not decrease the uncertainty in potential plant water sources due to the missing bulk soil water isotope data which was caused due to the little amount of cryogenically extracted bulk soil water. However, from the descriptive analysis of the collected isotope data could identify different end-members. We identified that soil water from recent rainfall as a first potential plant water source (Figure 5-8 and A4). Seeing the rather small range ($<1\text{‰ } \delta^{18}\text{O}$ and $<5\text{‰ } \delta^2\text{H}$) and partly overlapping isotopic composition of mobile soil water and ground water (Figure 5, 6, 8 and A4), we chose the median isotopic composition of soil water collected at -15 cm as a potential second plant water source. In addition, we observe in Figure 7 that the plant water samples drifted in time from the GMWL along the theoretical evaporation line of the soil water from antecedent rainfall. From this, we assume that the end point of the theoretical evaporation line can serve as proxy of the third potential water source if plants access tighter bound soil water -the immobile water. These three end-members were used in a simple three-component mixing model to explore and support which sources of water the rice plants potentially consumed during the different sampling days.

2) The construction of dual isotope space figures in which the plant and the different water sources are plotted together. In this way, it is easier the assess the isotope information per sampling period and seasons (Figure 7 and 8).

We agree and now included next to the isotopic composition of single rainfall samples also for each sampling day the mobile soil water and ground water data in the new Figure 7.

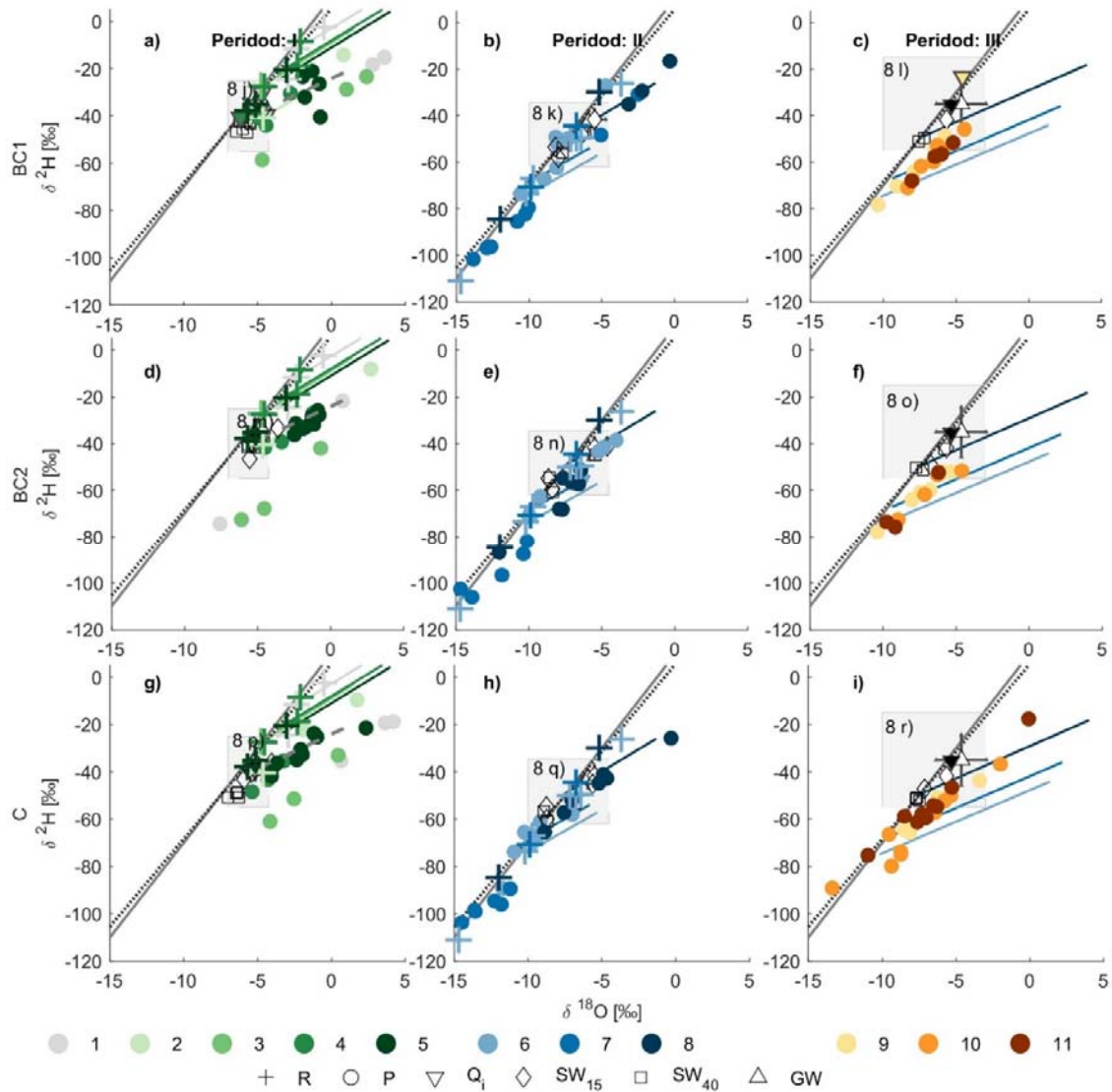


Figure 7 The dual isotope space with the isotopic composition of daily rainfall samples (crosses), plant water samples (circles), the calculated evaporation lines of residual rainfall and sampled soil water for the treatments BC1 (a-c), BC2 (d-f) and C (g-i) and periods I-III (columns). Colors indicate the different sampling days (note that lines in period III are blue because they have been obtained from samples taken in period II). The local meteoric line (black dotted line) and global meteoric water line (grey solid line) are indicated in all panels. The grey dashed lines (panel a, d and g) indicate the evaporation line of median soil water. Isotopic compositions of irrigation, soil water and groundwater vary within the grey shaded squares indicated as 8 j-r, and enlarged in figure 8 j-r.

3) Both water isotopes ($_2\text{H}$ and $_18\text{O}$) were determined for the plant and potential water sources, however, the results were only elaborated around ^{18}O . I would suggest you to describe both isotope ratios.

We included now also $\delta^2\text{H}$ in the text.

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