

Response to Anonymous Referee #2

We would like to thank Referee #2 for taking the time to review the manuscript. We have reproduced their comments, in blue, along with our responses in below.

To start with, the statistical analysis of the data should be improved, and that part of the Methods description should be elaborated and improved. As statistical method to assess the treatment effects in this study I recommend linear mixed effects models, see e.g. [Gueorguieva and Krystal, 2004; Crawley, 2009]. Crawley, M. J. (2009), *The R book*, 942 pp., John Wiley & Sons Ltd, Chichester. Gueorguieva, R., and J. H. Krystal (2004), Move over ANOVA, progress in analyzing repeated-measures data and its reflection in papers published in the archives of general psychiatry., *Archives of General Psychiatry*, 61, 310-317. Anon: Wiley: *The R Book*, 2nd Edition - Michael J. Crawley, [online] Available from: <http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470973927.html> (Accessed 14 September 2017), n.d. Thanks, we agree that a proper description of the statistics used was lacking and now have added a full description of our approach to the method section as suggested. We are not sure a mixed effect modelling approach is the best way forward for our data. We conducted a total of 18 incubations, with 6 incubations for each of the three levels (i.e. addition of δ_{iw-low} , δ_{iw-med} or $\delta_{iw-high}$ water) of water treatment. Whilst repeated measurements were made (i.e. the gas fluxes at different inlet conditions) on each incubation these are reduced to single parameters when regression coefficients are calculated. We test whether there are significant differences between soil properties or model parameters (determined from these coefficients) among water treatments. As such, we consider the 18 incubations to be independent for these tests. For this reason and as we are testing for differences between three population means (of the same factor / categorical independent variable i.e. δ_{iw} treatment), we used one-way analysis of variance. We chose not report statistical test of treatment effects for the gas flux data shown in Table 2 (and section 3.3), however, the reviewer is correct that a mixed effect modeling approach would be appropriate here. Hopefully the suggested improvements to the methods clarify this point.

P10 L23: *“Treatment summaries are reported as mean and standard deviation unless stated otherwise. A total of 18 incubations were conducted on sub-samples of same homogenised bulk soil. Six independently replicated incubations were conducted for each of the three δ_{iw} water treatments. Soil properties and model parameters were determined individually for each incubation as described above. Differences in soil properties and model parameters among δ_{iw} treatments, with statistical significance reported at $p < 0.01$, were tested through one-way analysis of variance with post-hoc comparison by Tukey's HSD (Crawley, 2007; Mendiburu, 2016). To do so, a given property or parameter was taken as the dependent variable and δ_{iw} treatment as the categorical independent variable.”*

I noted that the reference that is currently used in the Statistics part is missing on the reference list (Mendiburu, 2016).

The reference for Mendiburu was present but the new-line after the previous reference (Massman, 1998) was missing making it hard to see. We have corrected this, thanks.

Moreover, the Results section should be improved. In long parts many values are listed, e.g. means and error estimates for several parameters and treatments are spelled out in the text. I suggest to check which values are already given in the Tables, and to consider moving more of the values currently given in the text into Tables to refer to.

Following this advice we have removed duplicated numbers from the text and expanded Table 1.

Also, the authors are using many acronyms throughout the text. I find they are too many and this makes the text in parts hard to read. I suggest to reconsider which acronyms are central and to keep these, but consider to spell out certain variables (i.e. avoid too many acronyms). Alternatively, you might add a list of acronyms to the manuscript and refer to it repeatedly, to facilitate for the reader to look up the meaning of all acronyms during reading.

We agree that the manuscript makes use of several symbols that may need to be re-defined regularly to help the reader and, at the same time, we feel that the symbols used are vital to clearly relate to the methods without lengthening the text. For this reason we were careful to select consistent and logical symbols e.g. $\delta_{sw,ce}$ for soil water isotope composition determined following cryogenic extraction or $\delta_{sw,eq}$ for soil water isotope composition determined to be in equilibrium with CO_2 from gas flux measurements. However, we understand that following multiple symbols through a text can be difficult for the reader. In acknowledgement of this point, we have removed a number of less central symbols (e.g. δ_{atm} , $k_{iso,uncat}$, PTFE, GWC) and refer back to the meaning of important symbols at key points in the hope that this prevents the reader from having to search back through the text for first usage.

Please check as well that all acronyms are actually defined upon first use, and consider to even define acronyms that are common in your field but may not be obvious to all readers of the article (e.g. VPDBg and VSMOW-SLAP).

Done, thanks.

The same applies to the Tables and Figures, please include in footnotes or legend the meaning of the used acronyms (if you decide to keep them), with the goal that Figures and Tables can be understood independent of the text. As example I refer to the legend of Fig. 6, which contains four acronyms and is difficult to understand in its current form.

Following this good advice we have updated Table and Figure captions accordingly.

“Table 1: Soil properties by irrigation water (δ_{iw}) treatment. Means ($n = 6$) and standard deviations (in parentheses) for maximum soil depth (z_{max}), total porosity (f_t), and volumetric soil water content (q_w). Lower-case letters indicate significant differences (one-way analysis of variance and Tukey's HSD, $p < 0.01$) among δ_{iw} treatments.”

“Table 2: Gas exchange data by irrigation water (δ_{iw}) treatment at the three different incubation system inlet CO_2 (δ_b) conditions. Means and standard deviations (in parenthesis) for total CO_2 concentration in the bypass (C_b) and the chamber (C_a), the $\delta^{18}O$ of CO_2 in the bypass (δ_b) and the chamber (δ_a) and, the net flux of CO_2 (F_R) and its $\delta^{18}O$ signature (δ_R).”

“Table 3: Model solutions by irrigation water (δ_{iw}) treatment. Means ($n = 6$) and standard deviations (in parenthesis) for the piston velocity of CO_2 assuming a semi-infinite soil depth (v_{inv}), the piston velocity of CO_2 assuming a finite soil depth (\tilde{v}_{inv}), the apparent rate of ^{18}O exchange between CO_2 and soil water (k_{iso}), the effective diffusional fraction of CO_2 assuming a finite soil depth ($\tilde{\alpha}$), and the $\delta^{18}O$ of soil water in equilibrium with CO_2 as determined from gas flux measurements ($\delta_{sw,eq}$). Lower-case letters indicate significant differences (one-way analysis of variance and Tukey's HSD, $p < 0.01$) among δ_{iw} treatments.”

“Figure 1: Schematic of the system used to make gas exchange measurements. Alternate measurements of the concentration and $\delta^{18}O$ of CO_2 in chamber (C_a , δ_a) and bypass lines (C_b , δ_b) are made under inlet conditions that differ in terms of the $\delta^{18}O$ of CO_2 . “

“Figure 2: An example of the gas exchange measurement sequence, scanning sequentially calibration cylinders, the chamber line during a stabilisation period, calibration cylinders again, and finally the chamber and bypass lines, for the three different $\delta^{18}O$ of CO_2 delivered to the inlet of the incubation system (δ_b). In this case, the δ_b inlet conditions, whose changes are indicated by the vertical dashed lines, started with $\delta_{b,med}$ and ended with $\delta_{b,low}$. Symbols represent the calibrated average values and the dotted line is provided as a visual aid and does not correspond to raw 1-Hz data, (a) total CO_2 concentration and, (b) $\delta^{18}O$ of CO_2 .”

“Figure 3: Incubation depth profiles of the $\delta^{18}O$ of cryogenically extracted soil water ($\delta_{sw,ce}$), at intervals of 0-1, 1-2, 2-3, and 4-5 cm below the surface. Symbols and error bars indicate means and standard deviations by irrigation water (δ_{iw}) treatment and depth interval.”

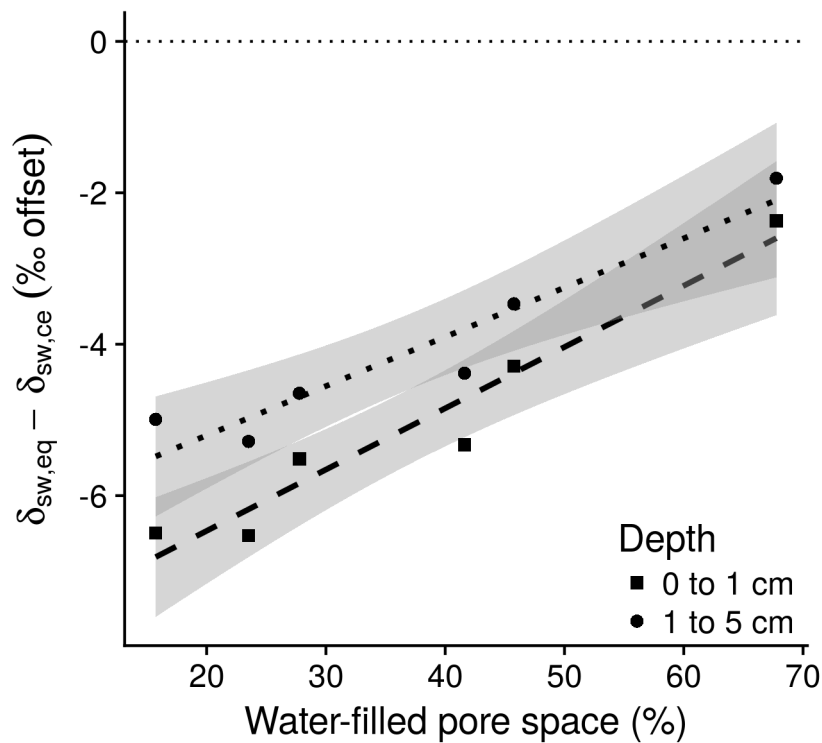
“Figure 4: Relationships between the $\delta^{18}O$ of soil-atmosphere CO_2 exchange (δ_R) and the $\delta^{18}O$ of CO_2 in the chamber line (δ_a) by irrigation water (δ_{iw}) treatment. Symbol shapes indicate measurements made at different inlet conditions (δ_b) that varied in terms of their $\delta^{18}O$ of CO_2 . Dashed lines indicate linear regressions for individual incubations.”

“Figure 5: Relationships between water-filled pore space and the difference between estimates of the $\delta^{18}O$ of soil water in equilibrium with CO_2 as estimated from gas flux measurements ($\delta_{sw,eq}$) and that estimated by cryogenic extraction ($\delta_{sw,ce}$) at depths of 0-1 cm (squares) and 1-5 cm (circles). Dashed lines and shaded areas indicate the linear regressions and associated 95 % confidence intervals for the two sampling depths.”

“Figure 6: Model relationships between the apparent rate of ^{18}O exchange (k_{iso}) between CO_2 and soil water and the $\delta^{18}O$ of soil water in equilibrium with CO_2 ($\delta_{eq,ce}$). These $\delta_{eq,ce}$ values were assumed from the depth averaged (0 to 5 cm) $\delta^{18}O$ of cryogenically extracted water for the incubations that received the $\delta_{iw,low}$ ($\delta^{18}O$ of -6.74 ± 0.03 ‰ VSMOW-SLAP) irrigation water treatment. Colours indicate the different responses for the same set of incubations at the three inlet conditions that differed by their $\delta^{18}O$ composition of CO_2 (δ_b).”

In Figure 5, please add confidence intervals to the regression lines. This may not be feasible in terms of clarity for Fig. 4, which contains many regression lines in one graph. In that case please add a note to the legend of Fig. 4 why confidence intervals are not shown.

We have not added these to Figure 4 for the reason indicated. We have added confidence intervals to Figure 5 and updated the caption accordingly.



“Figure 5: Relationships between water-filled pore space and the difference between estimates of the $\delta^{18}O$ of soil water in equilibrium with CO_2 as estimated from gas flux measurements ($\delta_{sw,eq}$) and that estimated by cryogenic extraction ($\delta_{sw,ce}$) at depths of 0-1 cm (squares) and 1-5 cm (circles). Dashed lines and shaded areas indicate the linear regressions and associated 95 % confidence intervals for the two sampling depths.”

Please add a reference for the assumed particle density [Linn and Doran, 1984]. Linn, D. M., and J.W. Doran (1984), Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils., Soil Science Society of America Journal, 48, 1267-1272

Done, thanks.

“Total porosity (ϕ) was calculated from bulk density assuming a particle density of 2.65 g cm^{-3} (Linn and Doran, 1984).”

“Linn, D. M. and Doran, J. W.: Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils, Soil Science Society of America Journal, 48(6), 1267–1272, doi:10.2136/sssaj1984.03615995004800060013x, 1984.”

P1/L11: Move the comma: “..., a group of enzymes that catalyse the hydration of CO_2 in soils and plants,...”

Done, thanks.

“To do so, the activity of carbonic anhydrases (CA), a group of enzymes that catalyse the hydration of CO_2 in soils and plants, needs to be understood.”

P5/20: “were monitored” (change from “was”)

Done, thanks.

“Relative humidity and temperature inside the humidifier were monitored using a small combined sensor and data-logger (Hydrochron, iButtonLink, LLC., USA).”

P9/L13: “R Development Core Team”

The citation provided by the citation function in R or indicated by the R-project website (<https://cran.r-project.org/doc/FAQ/R-FAQ.html#Citing-R>) uses 'R Core Team' rather than 'R Development Core Team'. We have added the relevant version information.

“All data processing and analysis was conducted in R version 3.3 (R Core Team, 2017).”