

Reply to reviewer 1 Matthias Beyer

In what follows, we respond to the individual comments and recommendations of reviewer 1, Matthias Beyer, MB. These responses are keyed to the specific comment by numbering, and are given in blue print, followed by indications of the changes made in the manuscript (in italics), and referring to the position in the original manuscript. Also, we revised again the entire manuscript for clarity, paying close attention to all of the reviewers' comments.

MB 1

Thank you for letting me review the manuscript 'The $\delta^{18}\text{O}$ ecohydrology of a grassland ecosystem – predictions and observations'. I enjoyed reading. In their work, the authors apply an $\delta^{18}\text{O}$ -enabled soil-plant-atmosphere transfer model in order to predict the dynamics of $\delta^{18}\text{O}$ in soil water, the depth of water uptake, and the effects of soil and atmospheric moisture on $\delta^{18}\text{O}$ -enrichment of leaf water in a grassland in southern Germany. In particular, they investigate the propagation of the $\delta^{18}\text{O}$ signal of rainwater through soil water pools, root water uptake and $\delta^{18}\text{O}$ enrichment of leaf water by tracing, predicting and validating $\delta^{18}\text{O}_{\text{soil}}$, $\delta^{18}\text{O}_{\text{stem}}$ and $\Delta^{18}\text{O}_{\text{leaf}}$. Finally, the authors test two models for describing $\Delta^{18}\text{O}_{\text{leaf}}$ at the canopy scale (the two-pool model or the Péclet model) and evaluate their performance.

We thank Matthias Beyer for the thorough and encouraging review and the detailed comments and recommendations that helped us much to improve the presentation of our work.

MB 2

Without doubt, this manuscript is well-prepared and written. The structure is clear, research questions are stated concisely, and the introduction provides a thorough overview on the topic. The graphics are suitable and well illustrated. I also agree to the authors that the model results are promising. The applied model MuSICA definitely seems capable of simulating ecohydrological processes including water isotopes. In my opinion, the hydrological and ecological community definitely needs a more integrated approach in modeling and investigating, and MuSICA seems a promising approach to that. I do not have major criticism on the manuscript, but a number of questions and comments that should be addressed in a revised version.

In summary those are: In general, I find that the discussion of the results needs to be more critical.

We revised the discussion thoroughly, considering all points raised by the reviewer (see responses to individual comments, below).

MB 3

Yes, the results are good for an uncalibrated model. BUT: Grass is (sorry for saying that) probably the simplest plant to model (homogeneous and short roots).

We are uncertain if modelling grass is inherently much simpler than modelling a non-grass species. For instance, the potential range of rooting depths of perennial grasses (and other grassland plants) can be very large (up to 6 m depth; cf. Schenk and Jackson, 2002), and grazing pressure (or defoliation frequency) can affect rooting depth very strongly (e.g. Klapp, 1971, Figure 43, page 81), providing scope for a large variability in rooting depth and depth of water uptake in different grassland systems.

In the revision we added a paragraph in the discussion pointing to this factor (see MB 9, below).

MB 4

Looking at the isotope results, the 20cm depth and also under dry circumstances does not really fit well – see R2. Hence, I would appreciate a more critical discussion, you have to highlight also the weaknesses that certainly still exist.

We believe that there is some misunderstanding here, and revised the text to eliminate any opportunity for such misunderstanding (again, see responses to individual comments, below).

In fact, the model performance for predicting $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm depth was really good, as was indicated by the close relationship of modelled and observed data ($R^2 = 0.79$) and the very small bias (MBE = 0.5‰; Table 2). Also, the observations and the model agreed rather well with respect to the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ (Figure 3): that relation was close in both the observed ($R^2 = 0.69$) and predicted data sets (0.65) and virtually unbiased at a depth of 7 cm, independently of soil water contents. Further, the predictions and observations agreed in that both indicated a poor relationship between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm, both in terms of scatter ($R^2 = 0.34$ for the observed and 0.17 for the model predicted relationships) and bias. On average, $\delta^{18}\text{O}_{\text{stem}}$ was ca 2‰ higher than $\delta^{18}\text{O}_{\text{soil}}|_{20}$, meaning that $\delta^{18}\text{O}_{\text{soil}}|_{20}$ did not agree with $\delta^{18}\text{O}_{\text{stem}}$. Thus, both the observations and the modelling independently indicated that water uptake must have occurred mainly from shallow depths (<20 cm).

In the revision, we worked through the text and relevant Table captions and Figure legends very carefully to enhance clarity and eliminate any ambiguity on model performance (see also response to MB 7, below).

The following main changes were made:

Abstract (P1 L18ff): “The model accurately predicted the $\delta^{18}\text{O}$ dynamics of the different ecosystem water pools, suggesting that the model generated realistic predictions of the vertical distribution of soil water and root water uptake dynamics. Observations and model predictions indicated that water uptake occurred predominantly from shallow (<20 cm) soil ...”

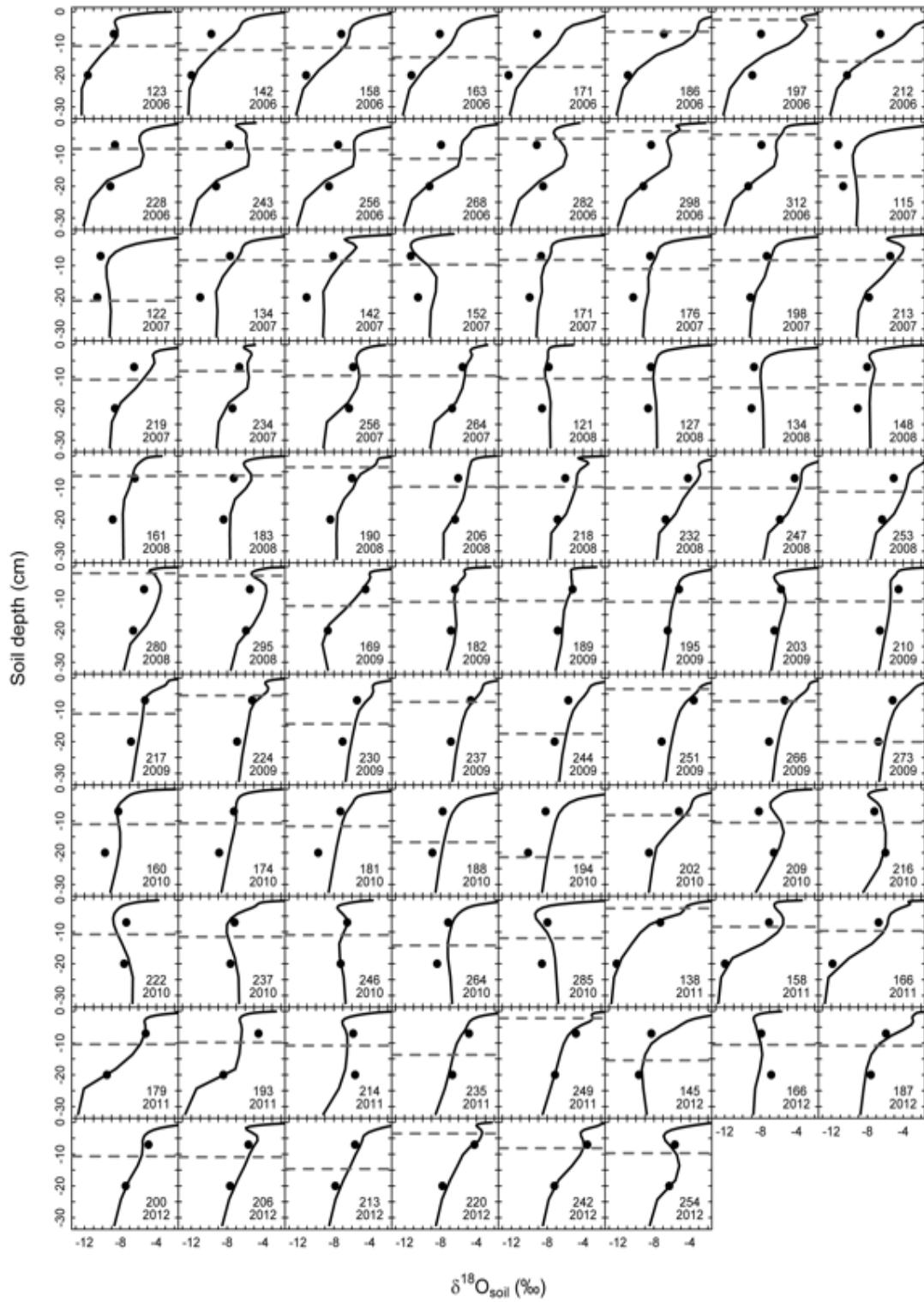
P11 L14ff: “Conversely, the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm was generally weak, exhibiting large scatter and a significant offset between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm for most of the data (Fig. 3c).”

P11 L22ff: “MuSICA simulations were based on this assumption and reproduced very similar relationships between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ as those observed at both depths, with similar R^2 , MBE and MAE (Figs. 2-3), thus showing a close agreement between observed and predicted data.

P14 L25ff: The comparison of observed $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ (Fig. 3a) strongly suggested that root water uptake occurred mainly at shallow depths (<20 cm) throughout the vegetation periods, largely independently of changes in SWC. That interpretation of observed data was based on comparison of $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at two depths (7 and 20 cm) only, which provides limited spatial resolution and cannot inform precisely on the depth of root water, if $\delta^{18}\text{O}_{\text{soil}}$ does not change monotonously with soil depth (Rothfuss and Javaux, 2017; Brinkmann et al., 2018). Such information can be improved by a locally-parameterized, physically-based, ^{18}O -enabled ecohydrological model, as shown here. For instance, the standard MuSICA runs (Fig. 3b) indicated near-monotonous increases of $\delta^{18}\text{O}_{\text{soil}}$ between 20 and 7 cm depth, matching well the observations in the majority of sampling dates (Fig. S13). Further, the simulations predicted a mean (uptake-weighted) depth of root water uptake at <15 cm, in 90% of all sampling dates, independently of SWC and observations of $\delta^{18}\text{O}_{\text{soil}}$. Support came also from the MuSICA sensitivity analysis (Fig. 6h) in showing that $\delta^{18}\text{O}_{\text{stem}}$ was well predicted by the model only when root length density was maximum at shallow soil depth. The potential range of rooting depths is large in grassland, depending on site, species, climatic and management effects (Schenk and Jackson, 2002; Klapp, 1971). So, why was root water uptake constrained to shallow depths in this drought-prone permanent grassland system? Several factors likely contributed: (1) the shallow top-soil overlying calcareous gravel (Schnyder et al., 2006), (2) the rapid shoot and root biomass turnover, that is associated with high phytomer dynamics leading to short leaf and root lifespan in intensively managed grassland (Schleip et al., 2013; Yang et al., 1998; Auerswald and Schnyder, 2009; Robin et al., 2010), (3) the high rates of shoot tissue (mainly leaves) losses that elicit a priority for assimilate (including reserve) allocation to shoot regeneration at the expense of the root system (e.g. Bazot et al., 2005), and (4) predominant placement of the root system near the soil surface dictated by the high need for nutrient interception and uptake (e.g. from excreta deposits), to compensate the high rates of nutrient losses due to grazing (Lemaire et al., 2000). Importantly, (5) in

a relatively high number of cases, the model predicted situations in which rainfall recharged mainly the top soil, while SWC at depths >20 cm remained low (e.g. June-end of year 2006, April–October 2007, or May-end of year 2008; Fig. S12; see also below). Principally, however, factors (2)–(4) alone can explain why shallow rooting depth is a typical feature of intensively grazed grasslands (Troughton, 1957; Klapp, 1971). Also, Prechsl ...”

Further, we added a supplemental figure (Figure S13), showing $\delta^{18}\text{O}_{\text{soil}}$ with soil depth as predicted by MuSICA (continuous lines) and mean uptake-weighted depth of root water uptake (dashed horizontal lines) on the different sampling dates. Closed circles: observations of $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm depth. Sampling date is given by DOY and year, in the lower right corner of each panel:



Legend of Fig. 3 (P29 L5ff):

“The R^2 , MBE and MAE for the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and the $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm depth were 0.69, 0.2‰ and 0.7‰ for the observed data (a) and 0.65, -0.2‰ and 0.7‰ for the predicted data (b). Conversely, the R^2 , MBE and MAE values for the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and the $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm depth were 0.34, 1.9‰ and 2.1‰ for the observed data (a) and 0.17, 1.8‰ and 1.9‰ for the predicted data (b).”

MB 5

Also, a total water balance is always a good means of validation and would be nice to have.

We agree with the reviewer. Unfortunately, we could not do a total water balance. E.g. we did not measure runoff (which was probably close to nil in this non-sloping pasture) and ground water recharge. The latter would have required installation of lysimeters, which was impractical on this intensively managed pasture. However, we did validate the model with latent heat flux data that were available from an eddy covariance station at the site, and we assessed the model's performance in predicting total plant-available water in the entire top soil by comparison with plant-available soil water modelling and data for the same site presented in Schnyder et al. 2006.

In the revision, we added a paragraph (P5 L23ff) stating: *“The model was validated with latent energy flux (LE) data obtained from an eddy covariance station (EC) at the site. According to that comparison (Fig. S1), MuSICA estimates were unbiased ($LE_{\text{MuSICA}} = 0.997 LE_{\text{EC}}$; $R^2 = 0.59$). Further, we compared MuSICA predictions of total plant-available soil water (PAW, mm) in the entire top soil with PAW modelling and data for the same site presented in Schnyder et al. (2006). For the 2007-2012 data, this yielded the relationship $PAW_{\text{MuSICA}} = 0.99 PAW_{\text{Schnyder et al. 2006}} + 7.8$ ($R^2 = 0.83$).”*

MB 6

The results section contains a lot of discussion (see detailed comments)

We eliminated discussion from the Results section following closely the reviewer's suggestions (see our answers to the specific comments below).

MB 7

Why was model not calibrated?

(This question is connected with point MB 5; see response above) We agree that we did not perform a classical calibration in the sense that the different model parameter values were statistically optimised. To do that we would have needed a greater number of hydrological measurements that we did not have (e.g. the dynamics of ground water recharge and soil water contents). The only instance where we did use parameter optimization (fine tuning) was in the case of the factors controlling ^{18}O enrichment of leaf water: mesophyll water content and night-time and minimal stomatal conductance (P9 L7-9), as well as the fraction of unenriched water in bulk leaf water. All other parameter values were based on measurements at the site, or – if such measurements were unavailable – on data from literature (as we explain). In that way we did ascertain realistic parameter values in this (otherwise) purely physically-based model. The fact that the model predicted well the $\delta^{18}\text{O}_{\text{soil}}$ at two different depths (that is a depth within the zone of most active root water uptake, 7 cm, and a depth just below that zone, 20 cm) did indicate strongly that the ensemble of parameters dictating soil water dynamics (including the spatial distribution of soil water uptake) in the zone of water uptake was described well by the model. This conclusion is further substantiated by the sensitivity analysis.

In the revision, we added the following short paragraph (see also response to MB 5) in P14 L15ff: *“The ability of the model to generate realistic predictions of the $\delta^{18}\text{O}$ dynamics at different depths in the soil (within the zone of most active root water uptake and just below that zone) suggests strongly that the ensemble of parameters dictating the spatio-temporal dynamics of soil water contents (including emptying and refilling dynamics) was described well in the model. That interpretation was also supported by the sensitivity analysis.”*

MB 8

Why was 2H not used? How was fractionation evaluated without 2H - did the authors simply use the offset of 18O from the LMWL? Is the model capable of modeling 2H as well? The dual-isotope space enables a more comprehensive understanding of processes. Also, it is more sensitive compared to 18O and since the authors did a sensitivity study, perhaps very useful. I don't say I expect that in a revised version, but I am interested on the authors opinion on that.

Yes, the MuSICA model is capable of simulating the $\delta^2\text{H}$ of soil water, xylem and leaf water. However, we elected to not include those data in the manuscript, as (1) we are primarily interested in the processes leading up to the $\delta^{18}\text{O}$ of cellulose, (2) we had noticed discrepancies in the model-data agreement for D/H that indicated fractionation (including a surface effect on D/H of soil water at the experimental site; Chen et al., 2016) that are currently not accounted for in the model. Hence, reporting both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ would have changed the focus of the paper and would have brought up additional questions (that we wish to investigate in a separate paper). Also (3), we did not want to overload the paper with extra figures and discussion.

In the revisions we added the following sentence (P5 L27ff): *Although the MuSICA model is capable of simulating $\delta^2\text{H}$ of water pools in the soil-plant system, we excluded those data in the manuscript, as (1) we are primarily interested in the processes leading up to the $\delta^{18}\text{O}$ of cellulose, (2) we had noticed discrepancies in the model-data agreement for D/H indicating fractionation (including a surface effect on D/H of soil water at the experimental site; Chen et al., 2016) that are currently not accounted for in the model, and (3) we did not want to overload the paper with extra figures and discussion. Issues of D/H fractionation of water including data from this experimental site will be addressed in a separate paper.*

MB 9

Having that said, I suggest minor revision. I am looking forward to see the manuscript published in HESS.

Detailed comments:

Abstract l.20: grazing pressure, but how about rooting depth? Grasses are shallow-rooted so any other uptake is not expected?!

As we mention above, the potential range of rooting depths of perennial grasses (and forbs) is very large and dependent on a wide range of factors including site conditions, species and management conditions (particularly grazing pressure or defoliation frequency). So, the predominance of water uptake from shallow depths is not necessarily a universal feature of grassland.

In the revision we added a phrase in the Abstract, P1 L20ff

"The model accurately predicted the $\delta^{18}\text{O}$ dynamics of the different ecosystem water pools, suggesting that the model generated realistic predictions of the vertical distribution of soil water and root water uptake dynamics. Observations and model predictions indicated that water uptake occurred predominantly from shallow (<20 cm) soil depths ..."

See also the detailed response to MB 4, above)

MB 10

l.20: respond to atmospheric moisture....does that mean leaves take up moisture from the atmosphere? (foliar uptake???)

Yes. Leaves exhibit bidirectional exchange of water vapour with the atmosphere, with a relative magnitude of the inward flux proportional to the relative humidity of the air, as we describe in the manuscript.

In the revision we changed the respective sentence to clarify the fact that it is actually the relative moisture ‘content’ of the atmosphere that drives the observed relationship. The sentence now reads (P1 L20): “ $\Delta^{18}\text{O}_{\text{leaf}}$ responded to both soil and atmospheric moisture *contents...*”

MB 11

1.21: two non-mixing pools: is that realistic or justified?

We see the point. Yes, the idea of two ‘non-mixing’ pools is a simplification, and unrealistic in the strict sense. The idea of having two discrete water pools in a leaf is the simplest conceptual model for explaining the observation that leaf water is usually less enriched than predicted by the Craig-Gordon model. The two-pool model is based on the notion that xylem and ground tissue are composed of unenriched water, whereas mesophyll cells are filled with evaporatively enriched water, implying constant fractions of unenriched and enriched leaf water (given full hydration of the leaves).

However, the reviewer is correct in questioning the realism of the ‘non-mixing pools’ idea, particularly in grasses that exhibit a continuous ^{18}O -enrichment towards the tip.

So, in the revisions we replaced the term ‘two non-mixing water pools’ by ‘two pool’ model characterized by constant proportions of unenriched and evaporatively enriched water. In the Abstract, this sentence now reads (P1 L20ff): “ $\Delta^{18}\text{O}_{\text{leaf}}$ responded to both soil and atmospheric moisture contents and was best described in terms of constant proportions of unenriched and evaporatively enriched water (two-pool model).”

MB 12

1.26: the second sentence is not well written/unconcise

The revised sentence now reads:

“*Meteoric waters impart their isotopic signal ($\delta^{18}\text{O}_{\text{rain}}$) to that of soil water ($\delta^{18}\text{O}_{\text{soil}}$), changing it as a function of refilling, exchange and percolation processes throughout the soil profile.*”

MB 13

1.29: explain better or provide citation – explain why do leaves fractionate

The revised sentence now reads:

“*The oxygen isotope composition of leaf water ($\delta^{18}\text{O}_{\text{leaf}}$) differs from that of the water taken up from the soil, as leaf water becomes ^{18}O -enriched due to evaporative effects and morpho-physiological controls (Barbour 2007).*”

MB 14

p. 2 1.14: ‘source water’ for plants would be soil or groundwater, but not xylem water as it is plant water already

We revised the sentence accordingly:

“*The isotopic composition of the water taken up by plants (henceforth termed $\delta^{18}\text{O}_{\text{stem}}$) can vary over time through changes in the depth of soil water uptake by roots or direct changes in soil water isotopic composition.*”

MB 15

p. 2 1.15/16: ‘summer’ and ‘winter’ should be related to the particular study area, these statements are not true for the whole earth....

We modified the sentence accordingly: “*For example, summer rains in continental Europe are usually isotopically distinct (^{18}O -enriched) relative to winter precipitation, generating intra-annual variations of $\delta^{18}\text{O}_{\text{soil}}$ ($\delta^{18}\text{O}$ of soil water) with soil depth.*”

MB 16

p. 2 l.29: ‘enrichment above....’ I know what you mean but this is written ambiguous – stem water can also be subject to fractionation under certain conditions. It should be more clearly expressed what is meant with this sentence.

We see the point.

Here we use the term $\delta^{18}\text{O}_{\text{stem}}$ to denote the $\delta^{18}\text{O}$ of the water taken up from the soil, and we define that term on first use. In what follows, we assume that there is no (relevant) further fractionation against ^{18}O , so that the water entering the leaf has the same $\delta^{18}\text{O}$ as that taken up by the root system as a whole.

We revised the annotated sentence, specifying that point: “*The mechanisms driving the isotopic enrichment of leaf water can be studied separately from those driving changes in $\delta^{18}\text{O}_{\text{stem}}$ by expressing the isotopic composition of leaf water as enrichment above $\delta^{18}\text{O}_{\text{stem}}$, i.e., $\Delta^{18}\text{O}_{\text{leaf}} = \delta^{18}\text{O}_{\text{leaf}} - \delta^{18}\text{O}_{\text{stem}}$, if the $\delta^{18}\text{O}$ of water entering the leaf is the same as that taken up by the root system as a whole.*

MB 17

p. 2 l.31: ‘many authors’ – could you provide some citations, please?

We added a citation to a pertinent review: Cernusak et al. 2016.

MB 18

p. 3 ll.2-14: this is well written!

Thank you!

MB 19

p. 3 l.15: is this relevant for grasslands only?

Actually, there is no reason to believe that this is only relevant for grassland.

So, we deleted ‘grassland’.

MB 20

p.4.1.5: please review this sentence and provide more information...which species, which soil depths, what exactly is meant with ‘growing season’

We added the requested info.

The paragraph now reads: “*To explore these questions we compared predictions from the ^{18}O -enabled soil-plant-atmosphere model MuSICA (Ogée et al., 2003; Wingate et al., 2010; Gangi et al., 2015) with those observed in a unique, multi-annual data set (7 years) of growing season (April to November), biweekly samplings and $\delta^{18}\text{O}$ analysis of soil water (at 7 and 20 cm depth), stem and midday leaf water, atmospheric water vapour, along with rainfall amount and $\delta^{18}\text{O}_{\text{rain}}$ data. The experimental site (Schnyder et al., 2006) was an intensively grazed *Lolio-Cynosuretum* (Williams and Varley, 1967; Klapp, 1965) community with *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, *Taraxacum officinale*, and *Trifolium repens* as the main species. Vegetation samples were taken as mixed-species samples, as described below.*

MB 21

p.5.18: though you cite a paper on the cryogenic system you use, it would be nice to specify temperature and extraction time here

We revised the sentence as follows:

“All samples were stored in a freezer at approx. -18°C until water extraction. Water was extracted for two hours using a cryogenic vacuum distillation apparatus with sample vials placed in a water bath with a temperature set to 80°C (Liu et al., 2016).”

MB 22

p.6.ll. 1 & 2-7: These information belong together, I'd suggest to either put the first part down or the second up

We followed the recommendation and revised the paragraph as follows:

“MuSICA was forced by half-hourly values of meteorological data and $\delta^{18}\text{O}$ of water vapour ($\delta^{18}\text{O}_{\text{vapour}}$) and rainwater ($\delta^{18}\text{O}_{\text{rain}}$). Wind speed, precipitation, air temperature, relative humidity and air pressure data were obtained from the Munich airport meteorological station, located at about 3 km south of the experimental site. Radiation was calculated as the mean of two weather stations located 10 km west and 12 km east of the experimental site. CO_2 concentration was measured at the site by an open-path infrared $\text{CO}_2/\text{H}_2\text{O}$ gas analyser (LI-7500, LI-Cor, Lincoln, USA). For $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$, observations at the experimental site were used whenever available. Otherwise $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ estimates were obtained from globally-gridded reconstructions derived from the isotope-enabled, nudged atmospheric general circulation model IsoGSM (Yoshimura et al., 2011). The IsoGSM-predicted $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ at the grid point relevant to our site were first corrected for their offset with observed data, as predictions were found to be more enriched by 2‰ and 1.3‰ on average compared to the $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ measured at the site (Figs. S2–S4).”

MB 23

p.7 l. 33: based on what was the beta distribution assumed (based on previous research or citation)

The beta distribution was shown to provide a good description of the vertical distribution of root-length-densities (e.g. Sadri et al., 2018).

We added a reference to Sadri et al. (2018).

MB 24

p.10.l. 2: Why does the ratio need to remain 1.6?

In their review, Medlyn et al. (2002) found a close relationship between the potential rate of electron transport (J_{max}) and the maximum rate of carboxylation (V_{cmax}) for a broad range of crop, broadleaf and coniferous species. The slope of that regression was 1.6. Based on that study, we assumed a constant $J_{\text{max}}/V_{\text{cmax}} = 1.6$ also in our work (see Supplement, Table S1).

In the revision, we added the citation to Medlyn et al. (2002) in the main text. The sentence now reads: *“ V_{cmax} and J_{max} were altered in tandem to keep the ratio $J_{\text{max}}/V_{\text{cmax}}$ at 1.6 (Medlyn et al., 2002), the same as in the standard simulation (Table S1).”*

MB 25

p.10.ll. 4-6: Perhaps that fits better to 2.4.1 isoforcing

We revised the text in section 2.5 that was misleading, to clarify that the sentence relates to the sensitivity analysis and not to the isoforcing for the standard simulation.

That sentence now reads *“In addition, we investigated the effect of using uncorrected IsoGSM-predicted $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data instead of local isotopic data (gap-filled with offset-corrected IsoGSM data; see 2.4.1) for the isoforcing of MuSICA. This served to illustrate the usefulness of having local rainwater $\delta^{18}\text{O}$ data.”*

MB 26

p.10.l21: Was predicted soil water content validated somehow?

Yes, we obtained a good agreement between predictions of soil water content with MuSICA with predictions obtained using the approach described by Schnyder et al. (2006) for the same site.

See response to MB 5, above

MB 27

p.11.1.29: in the way that (word missing)

We inserted '*in the way that*'.

MB 28

p.11: paragraph 3.4 contains a lot of discussion, I suggest reviewing and removing some of the 'judging' (e.g. last sentence or 1.29/30)

We revised the paragraph, accordingly.

MB 29

p.12.1.21: MLR does not appear in the methods/statistics

We added in the Statistics section: "*Simple and multiple linear regression analyses and student's t tests were performed in R, version 3.4.2 (R Core Team, 2017) and RStudio, version 1.1.383 (RStudio Team, 2016).*"

MB 30

p.12.1.23: weakly significant? I think this should be rephrased ! significant or not

The P values for the predicted and observed regressions lay between 0.05 and 0.1, i.e. close to significant. Thus, the sentence was rephrased as follows: "*The interaction effect of air relative humidity and SWC was close to significant for both observed ($P = 0.080$) and predicted ($P = 0.073$) $\Delta^{18}\text{O}_{\text{leaf}}$ (Table 4).*"

MB 31

p.12. paragraph 3.5.: the authors mix VPD and relative humidity quite a lot here, which makes this chapter hard to read. I suggest restructuring and rephrasing of this chapter (though the results completely make sense)

We agree and restructured the paragraph.

The new text now reads: "*Multiple regression analysis demonstrated significant effects of air relative humidity ($P < 0.01$) and SWC ($P < 0.05$) on both observed and predicted $\Delta^{18}\text{O}_{\text{leaf}}$ (Table 4). $\Delta^{18}\text{O}_{\text{leaf}}$ increased with decreasing air relative humidity and SWC (Figs. 4a, b and 5a, b). The interaction effect of air relative humidity and SWC was close to significant for both observed ($P = 0.080$) and predicted ($P = 0.073$) $\Delta^{18}\text{O}_{\text{leaf}}$ (Table 4). The effect of dry soil conditions on $\Delta^{18}\text{O}_{\text{leaf}}$ was most evident at low air humidity (Figs. 4a, b and 5a, b) and was connected with a decrease of canopy conductance (g_{canopy}) (Fig. 5c).*

The modelled dependence of transpiration on air VPD (the climatic driver of transpiration) was strongly modified by SWC (Fig. 4c). High air VPD drove high transpiration rates only under wet soil conditions ($\text{SWC} \geq 0.25$)."

MB 32

p.131 4-10: Discussion

p.13. 1.26-32: This sounds more like a conclusion

This paragraph is summarizing the main observations on model-data agreement. We would like to retain it, as it is.

MB 33

p.14. 1.5: quite

We removed 'quite'

MB 34

p.14. 1.6-7: suggest rephrasing: 'likely result from sampling effects and analytical error'

We agree and rephrased the sentence as follows: "*The greater scatter in the observed relationship between $\Delta^{18}\text{O}_{\text{leaf}}$ and relative humidity compared to predictions (Fig. 4) likely resulted partly from sampling effects and error.*"

MB 35

p.14. 1.12-23: I agree, but also it should be clear that grass with a fairly uniform uptake depth right below surface is probably the easiest of plants to model. This is not a criticism but would be interesting how the model performs for different plant types.

We agree, in principle. Yes, it would be extremely interesting to also test the model for its performance with different biomes in different site conditions, exploring also especially systems that include deep-rooted species.

MB 36

4.2: I am not sure if this deserves an own chapter. I believe that it is true that the grass takes the water mainly from the upper depths but considering the characteristic shape of soil water isotope profiles at the surface (enrichment and subsequent decrease of isotope values towards a constant value), the used resolution of only 2 depths might not reveal true uptake patterns. Also see Rothfuss and Javaux, 2016.

We see the point, and the caveat. We are aware of the fact that the soil water $\delta^{18}\text{O}$ values from only two depth positions do not necessarily reflect the total range of $\delta^{18}\text{O}$ expected for the entire soil profile. Nevertheless, the model simulations generated a detailed prediction of how $\delta^{18}\text{O}$ varied along the profile. For the sampled depth, the predictions matched the observations generally well. We added a supplemental figure (Figure S13) showing the predicted soil water $\delta^{18}\text{O}$ profiles (see response to MB 4, above). The most extreme (positive) values were predicted for the uppermost 1-2 cm of the soil (Fig. S13), as a consequence of evaporative ^{18}O enrichment at the soil surface. The model predicted very little root water uptake in that zone (Fig. S12).

The $\delta^{18}\text{O}$ of soil water at 7 cm was greater (i.e. more enriched) than the $\delta^{18}\text{O}$ at 20 cm for 79 out of 86 cases, i.e. for more than 90% of the dataset. In line with that, the model mostly predicted a decrease of $\delta^{18}\text{O}$ between 7 and 20 cm, which was monotonous for a large part of the dataset (new Figure S13). Even if the decrease was not monotonous (e.g. in late summer/autumn of 2006), the highest and lowest $\delta^{18}\text{O}$ values were still found in the upper and lower profile, respectively. Hence, at least the qualitative assessment that the roots take up the water from the shallow horizon was still valid in those cases.

On 12 days, $\delta^{18}\text{O}_{\text{soil}}$ was predicted to be quite constant from approx. 5 cm to the bottom of the profile. In those specific cases, additional soil samples between 5 and 37 cm would not have had additional value with regard to inferring the depth of water uptake by comparing $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$. On another 6 days in 2008 and 2010 (e.g. DOY 209 and 285 in 2010), the uptake depth could not be unambiguously inferred by comparing $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$. Considerable rainfall had occurred in the two weeks preceding those sampling days (e.g. 61 litres of rain during DOY 203 to 208 of 2010), creating non-monotonous isotopic profiles (e.g. an S-shaped profile on DOY 209 of 2010). In those cases, the model predictions, which were solely based on hydraulic properties of the soil, root architecture and evaporative demand, and not on observed $\delta^{18}\text{O}_{\text{soil}}$ data, can help to deduce the root water uptake depth. For day 209 in 2010 for example, the model predictions indicated that the average mass-weighted root water uptake depth was located at 10.5 cm (dashed horizontal line in Fig. S13 for that DOY).

We revised this chapter thoroughly, paying close attention to the reviewers' concerns. See response to MB 4, above.

MB 37

p.15. 1.26-27: 'online transpiration isotope method' this appears here for the first time?

Yes. These data help us in the discussion, in that they provide supporting evidence for the two-pool model also for individual grass species (that were part of the codominant species in our grassland community).

The methods and results of these supplementary experiments with *Lolium perenne* and *Dactylis glomerata* are described in the Supplement. The citation to that description (Notes S2) was missing and is now added to the revised manuscript:

*"We did not know if putative between-species differences in leaf water dynamics and associated ^{18}O -enrichment, or any other morpho-physiological effects e.g. associated with leaf aging, could have led to a missing correlation between the proportional difference between measured leaf water ^{18}O -enrichment and that predicted by the Craig-Gordon model ($1 - \Delta^{18}\text{O}_{\text{leaf}} / \Delta^{18}\text{O}_{\text{e}}$) and transpiration rate. For these reasons, we explored this question with separate studies of *L. perenne* and *D. glomerata*, two species that also formed part of the present grazed grassland ecosystem. Again, these studies found no evidence for a Péclet effect, and supported the two-pool model, as there was no relationship between the proportional difference between measured leaf water enrichment and that predicted by the Craig-Gordon model ($1 - \Delta^{18}\text{O}_{\text{leaf}} / \Delta^{18}\text{O}_{\text{e,ss}}$) and transpiration rate in either *L. perenne* plants grown in a controlled environment at different relative humidities and water availabilities, or *D. glomerata* leaves measured using an online transpiration isotope method (Notes S2 and Figs. S14-15)."*

MB 38

p.16 1.9-11: I like this chapter, but the last sentence does not make sense – why compare and justify grass species with a study on non-grass-species?

We do not wish to justify our data by comparison with non-grass species. However, it is interesting and important to note that the range of proportional differences between measured leaf water ^{18}O enrichment and that predicted by the Craig-Gordon model (ϕ) is very similar in grasses and dicots.

We revised the faulted sentence, which now reads: *"Considering a similar effect of vein removal would move our observed ϕ to about 0.2. Such a value of ϕ for grasses is very similar to the mean ϕ reported for a wide range of non-grass species by Cernusak et al. (2016)."*

MB 39

Conclusions: An experienced and known Professor once gave me the advice 'A good paper doesn't need a conclusion – the reader draws it him/herself.' The authors should decide themselves, but I feel emphasizing some key points in the manuscript/abstract a bit more would be sufficient without conclusion.

We deleted the Conclusions, and emphasized key points, as documented above.

MB 40

Fig. 3: As stated above, the model does not work that well for ^{18}O . I think this needs to be discussed thoroughly

See our response to MB 4 (above).

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Reply to reviewer 2

In what follows, we respond to the individual comments and recommendations of reviewer 2, R2. These responses are keyed to the specific comment by numbering, and are given in blue print, followed by indications of the changes made in the manuscript (in italics), and referring to the position in the original manuscript. Also, we revised again the entire manuscript for clarity, paying close attention to all of the reviewers' comments.

R2 1

Hirl and coauthors present an impressive data set of seven years of isotopic observations in a grassland and an equally impressive modelling effort of the data.

We thank the reviewer for the encouraging and thought-provoking comments that helped us much to improve the presentation of our work.

R2 2

The interpretation of the data is regrettably only discussing the isotopes and gives very little insight into the water fluxes of the ecosystem.

Yes, our results and interpretations centre on ^{18}O of water in the different ecosystem components, although we do present model predictions of canopy conductance and transpiration as a function of soil water content and leaf-to-air vapour pressure deficit (Fig. 4c and 5c), we compare measurements and predictions of latent heat flux (Fig. S1), and we make predictions of the soil depth distribution of water contents and root water uptake (Fig. S12). This approach was dictated by the main focus of the work that consisted in systematically evaluating our (eco)system-scale understanding of the propagation of the $\delta^{18}\text{O}$ signal of rainwater through soil water, root water uptake and ^{18}O enrichment of leaf water (as specified in the Introduction, P3 L30ff), by comparison of model predictions and observations. In that sense, our work is 'restricted' to the ^{18}O -ecohydrology of the system, as we explore how the different hydrological properties of the system (given by the parametrization of the model) dictate the dynamics of $\delta^{18}\text{O}$ of water with depth in the soil, water taken up by the vegetation, and enrichment in the leaves. We believe that this is the most novel aspect of this work, and also the topic that we can support/validate/evaluate best with observations. Thus, our paper demonstrates how knowledge of $\delta^{18}\text{O}$ of distinct water pools can help us to assess the ability of a locally-parameterized ^{18}O -enabled mechanistic soil-plant-atmosphere model in predicting the hydrology of a system. For instance, the fact that the ^{18}O -enabled hydrology inside MuSICA predicted well the observed ^{18}O -dynamics at different depths in the soil and in the water taken up by the root system indicates strongly that the ensemble of model parameters also predicted well the spatio-temporal dynamics of soil water contents (including emptying and refilling dynamics) and depth distribution of root water uptake. We hope that this approach – when developed further – can also be helpful later on for the ecohydrological interpretation of $\delta^{18}\text{O}$ in biological archives (e.g. $\delta^{18}\text{O}_{\text{cellulose}}$ extracted from Park Grass Experiment hay samples originating from the last century).

To address the point in the revision, we

- added the definition of the term ^{18}O -ecohydrology and its' objectives and potential (P2 L5). *"This science, that explores relationships between the spatio-temporal dynamics of water in the soil-vegetation-atmosphere system with help of the dynamics of $\delta^{18}\text{O}$ of water in its different components, may be termed ^{18}O ecohydrology."*
- improved and expanded the discussion/interpretation of soil water dynamics and root water uptake (see below, and responses to reviewer 1, Matthias Beyer).

R2 3

For example, if main water uptake is always at 7 cm depth even when this layer falls dry, then ecosystem transpires probably less than possible during this times because it would have access to more water in deeper soil. How is the ecosystem reacting? Is it shutting down the stomata? Is it

changing its carboxylation capacity and stomata close thence? Or both? And why would a grassland do this? I guess it is well established in trees that they would harvest deeper soil water.

Yes, these are important points, that we address in 4.2 (revised, see below). See also responses to MB and relevant changes made, above.

Being restricted to only 2 depths, the spatial resolution of our observations of $\delta^{18}\text{O}_{\text{soil}}$ is limited, and there are methodical issues on the precision for estimation of the depth of root water uptake from such observations alone. Here, the (locally parameterized) hydrological model inside MuSICA does help. This predicted that root water uptake occurred over a broader zone (Fig. S12), with a mean (uptake-weighted) depth of root water uptake above a soil depth of 15 cm in 90% of all sampling dates (new Fig. S13).

We had no observations of stomatal conductance and carboxylation capacity, that would allow us to address their responses to drying soil. However, the model did consider an effect of soil drying on stomatal conductance (dependent on predawn leaf water potential) (P7 L22-24). The predicted effect of that is displayed in Fig. 5c. The sensitivity analysis did show that predictions of $\delta^{18}\text{O}_{\text{soil}}$ at the different depths was responsive to stomatal conductance. Therefore, the generally good agreement between observed and predicted $\delta^{18}\text{O}_{\text{soil}}$ did suggest that the ensemble of (photosynthetic and hydrological) model parameterization predicted the spatio-temporal variation in SWC and root water uptake quite well.

Interestingly, the model also predicted that SWC were occasionally lower below 25 cm than above that depth, particularly when rainfall recharged the top soil, but was insufficient to recharge the soil at greater depths (Fig. S12). Such phenomena occurred relatively frequently in the second half of the growing season. That fact could contribute additionally to explain why root water uptake occurred mainly from shallow soil depths (i.e. <20 cm below soil surface).

Certainly, the shallow root distribution also dictated a shallow depth of root water uptake. That shallow root distribution probably resulted from morpho-physiological constraints, particularly in the grasses and white clover (which comprised about 90% of the total pasture vegetation): in these species, adventitious roots compose virtually the entire root system, and root turnover is rapid and connected with leaf turnover at phytomere level (Yang et al., 1998; Robin et al., 2010) and assimilate supply to roots is reduced when grazing pressure is high (e.g. Bazot et al., 2005). In addition, the extremely high nutrient demand of frequently-defoliated vegetation is another factor that contributes to explain the formation and maintenance of a very shallow root system, as virtually all nutrient returns (mainly excreta from the grazing cattle) occur superficially.

We revised the entire manuscript for clarity concerning the above issues and, particularly, revised rigorously the first part of section 4.2, which now reads:

“The comparison of observed $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ (Fig. 3a) strongly suggested that root water uptake occurred mainly at shallow depths (<20 cm) throughout the vegetation periods, largely independently of changes in SWC. That interpretation of observed data was based on comparison of $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at two depths (7 and 20 cm) only, which provides limited spatial resolution and cannot inform precisely on the depth of root water, if $\delta^{18}\text{O}_{\text{soil}}$ does not change monotonously with soil depth (Rothfuss and Javaux, 2017; Brinkmann et al., 2018). Such information can be improved by a locally-parameterized, physically-based, ^{18}O -enabled ecohydrological model, as shown here. For instance, the standard MuSICA runs (Fig. 3b) indicated near-monotonous increases of $\delta^{18}\text{O}_{\text{soil}}$ between 20 and 7 cm depth, matching well the observations in the majority of sampling dates (Fig. S13). Further, the simulations predicted a mean (uptake-weighted) depth of root water uptake at <15 cm in 90% of all sampling dates, independently of SWC and observations of $\delta^{18}\text{O}_{\text{soil}}$. Support came also from the MuSICA sensitivity analysis (Fig. 6h) in showing that $\delta^{18}\text{O}_{\text{stem}}$ was well predicted by the model only when root length density was maximum at shallow soil depth. The potential range of rooting depths is large in grassland, depending on site, species, climatic and management effects (Schenk and Jackson, 2002; Klapp, 1971). So, why was root water uptake constrained to shallow depths in this drought-prone permanent grassland system? Several factors likely contributed: (1) the shallow top-soil overlying calcareous gravel (Schnyder et al., 2006), (2) the rapid shoot and root biomass turnover, that is associated with high phytomer dynamics leading to short leaf and root lifespan in intensively managed grassland (Schleip et al., 2013; Yang et al., 1998; Auerswald and Schnyder, 2009; Robin et al., 2010), (3) the high rates of shoot tissue (mainly leaves) losses that elicit a priority for assimilate

(including reserve) allocation to shoot regeneration at the expense of the root system (e.g. Bazot et al., 2005), and (4) predominant placement of the root system near the soil surface dictated by the high need for nutrient interception and uptake (e.g. from excreta deposits), to compensate the high rates of nutrient losses due to grazing (Lemaire et al., 2000). Importantly, (5) in a relatively high number of cases, the model predicted situations in which rainfall recharged mainly the top soil, while SWC at depths >20 cm remained low (e.g. June-end of year 2006, April–October 2007, or May-end of year 2008; Fig. S12; see also below). Principally, however, factors (2)–(4) alone can explain why shallow rooting depth is a typical feature of intensively grazed grasslands (Troughton, 1957; Klapp, 1971). Also, Prechsl et al. (2015) did not find an ...”

R2 4

Are any of the other variables telling me something about the ecophysiology of the plants or the ecohydrology of the ecosystem? Are leaf water isotopes telling me something? They tell me at least that there is nighttime conductance. Is there also nighttime transpiration? Anything else?

In the main, the ecophysiology of the plants and the ecohydrology of the ecosystem is reflected in the parameterization of vegetation and soil in MuSICA (Methods S2, Table S1, Figure S5, S6, S8), with many parameter values obtained from local measurement. The spatio-temporal dynamics of root water uptake (Fig. S12), and canopy conductance (Fig. 5c) and transpiration rate (Fig. 4c) at midday predicted by MuSICA are a result of that parameterization.

And yes, the diurnal $\delta^{18}\text{O}_{\text{leaf}}$ data indicate that stomates were not completely closed during the night (P7 L18–19), a factor that was reflected in the parameterization of MuSICA (Table S1). Yet, predicted night-time transpiration (estimated by latent energy flux) was always very low, in agreement with the eddy flux data (Fig. S1) and the generally high nocturnal relative humidity.

We did not have the detailed ecophysiological and ecohydrological observations to validate those specific predictions. However, we did validate MuSICA for the evapotranspiration (i.e. latent heat flux) predictions, and estimations of plant-available soil water in the entire top-soil (see also changes made in response to reviewer 1).

Most importantly, the good agreement between observed and predicted $\delta^{18}\text{O}$ in soil (at 7 and 20 cm depth), stem and leaf water does indicate that the model described the ecohydrology of the grassland system well.

In the revision, we added several sentences and phrases, clarifying those points (see also responses to reviewer 1):

P5 L27ff: “The model was validated with latent energy flux (LE) data obtained from an eddy covariance station (EC) at the site. According to that comparison (Fig. S1), MuSICA estimates were unbiased ($LE_{\text{MuSICA}} = 0.997 LE_{\text{EC}}$; $R^2 = 0.59$). Further, we compared MuSICA predictions of total plant-available soil water (PAW, mm) in the entire top soil with PAW modelling and data for the same site presented in Schnyder et al. (2006). For the 2007–2012 data, this yielded the relationship $PAW_{\text{MuSICA}} = 0.99 PAW_{\text{Schnyder et al. 2006}} + 7.8$ ($R^2 = 0.83$).”

P7 L20ff: “Although the diurnal pattern of $\delta^{18}\text{O}_{\text{leaf}}$ (Fig. S7) indicated some nocturnal stomatal conductance, the model generally predicted very low nighttime transpiration, in agreement with the eddy flux data (Fig. S1) and the generally high nocturnal relative humidity.”

P 14 L12ff: These ecohydrological processes are described explicitly in MuSICA, and agreement between observations and predictions of $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm depth indicates that MuSICA is capable of simulating these ecohydrological processes including ^{18}O of the different water pools.

And P15 L4ff: “Predictions of $\delta^{18}\text{O}_{\text{soil}}$ particularly below the main zone of most water uptake, at 20 cm, were influenced markedly by estimates of LAI...”

R2 5

I think, therefore, that the claim in the conclusions that the "work highlights the usefulness of mechanistic ^{18}O -enabled modelling for explorations and quantitative analyses of the ecohydrology of ecosystems." is premature because only point (2) of the three points, i.e. root water uptake is actually ecohydrology of the ecosystem. The other points are about ^{18}O ecohydrology, as the title of the paper suggests.

We understand the criticism, which is – we believe – partly due to our omission of a clear definition of ^{18}O -ecohydrology, and the objectives of its application in the present context.

In the revision, we added the definition in the Introduction. Here, we employed the ecohydrological model implemented in MuSICA to predict the $\delta^{18}\text{O}$ of water at different soil depths, the $\delta^{18}\text{O}$ of water taken up from the soil, and the ^{18}O -enrichment in leaves. In that we also evaluated several methodical/conceptual, ^{18}O -ecohydrological uncertainties impacting on such predictions, such as (1) the choice of the water vapour effective diffusivity in the soil (Moldrup vs Penman), (2) the source of the rain water and atmospheric vapour $\delta^{18}\text{O}$ (local data vs IsoGSM estimations), and (3) alternative leaf water- ^{18}O -enrichment models (two-pool vs Péclet). The capability of the model to predict the $\delta^{18}\text{O}$ of the different water pools then indicates that the model is equally capable to predict the different ecohydrological processes (that underlie the $\delta^{18}\text{O}$ predictions and observations).

Also, we revised all text carefully to eliminate any opportunity for misunderstandings. In particular, we highlight that a physically-based ^{18}O -enabled ecohydrological model (as implemented in MuSICA) can provide insight in ecohydrological processes, such as the spatio-temporal dynamics of soil water and root water uptake, and transpiration or canopy/stomatal conductance. Concerning the latter, we find it interesting that dry soil conditions (under similar atmospheric conditions) led to increased ^{18}O -enrichment (on average) in both the observed and predicted data, although evidence for a Péclet effect was missing in our data.

In the revision, we made the following main corrections, additions and deletions:

Abstract

P1 L16: "Using the ecohydrology part of a physically-based, ^{18}O -enabled soil-plant-atmosphere transfer model (MuSICA), we evaluated our ability to predict the dynamics ..."

P1 L18: "The model accurately predicted the $\delta^{18}\text{O}$ dynamics of the different ecosystem water pools, suggesting that the model generated realistic predictions of the vertical distribution of soil water and root water uptake dynamics. Observations and model predictions indicated that water uptake occurred predominantly from shallow (<20 cm) soil depths ..."

Introduction

P2 L5: "This science, that explores relationships between the spatio-temporal dynamics of water in the soil-vegetation-atmosphere system with help of the temporal dynamics of $\delta^{18}\text{O}$ of water in its different components, may be termed ^{18}O ecohydrology".

Conclusion

We deleted the Conclusions section (see also response to reviewer1, MB 39)

R2 6

I have to admit that I had problems with the sensitivity analysis. Firstly, the mean difference is not a good measure. Differences can cancel out even when the model reacts strongly to a change. Most people use variance, standard deviation or root mean square error to avoid this. I guess that would be something like the error bars in Fig. 6. Secondly, one can of course use "arbitrary" ranges of model parameters to look at the output range, but then one cannot compare anymore the output ranges between the different parameters as done in Fig. 6. One wants to disturb each parameter similarly. So a derivative would probably be a good idea, or an elasticity.

We understand the point raised by the reviewer. We realize that our description of the sensitivity analysis and of Fig. 6 lacked some precision, and we improved that in the revision (see below).

We like to emphasize that our sensitivity analysis presents two types of (sensitivity) information/variables: (1) the mean sensitivity to a change of a parameter value (upper or lower value) on the metric of interest (e.g. $\delta^{18}\text{O}_{\text{leaf}}$), in relation to the standard simulation, as shown on the x-axis as

‘mean sensitivity’, and (2) the standard deviation of the sensitivity (given by the error bar). The standard deviation captures the variability of the response to a parameter change between the individual sampling occasions. If cancelling effects result from the change of a parameter value, resulting in a mean sensitivity close to zero, that cancelling behavior is revealed by the (size of the) standard deviation of the sensitivity (e.g. the effect of the upper parameter value on $\delta^{18}\text{O}_{\text{leaf}}$ in panel 6h). Panel 6a reports a very different type of behavior, as changing the parameter value caused no cancelling effects on $\delta^{18}\text{O}_{\text{leaf}}$ (as was indicated by the small standard deviation of the sensitivity), but a strong change in the mean sensitivity for $\delta^{18}\text{O}_{\text{leaf}}$. So, there were instances where changes of parameter values caused a ‘general’ effect (causing a positive or negative mean sensitivity) and instances where there were strong cancelling effects (leading to a large standard deviation of the sensitivity). Both types of sensitivities can be gleaned from our presentation of parameter sensitivities.

Thus our sensitivity analysis revealed four different types of sensitivities: (a) strong mean sensitivities, with no or little cancelling (e.g. $\delta^{18}\text{O}_{\text{leaf}}$ in panel 6a), (b) mean sensitivities combined with strong cancelling effects (e.g. $\delta^{18}\text{O}_{\text{leaf}}$ in panel 6c), (c) no mean sensitivities resulting from strong positive and negative cancelling effects (e.g. $\delta^{18}\text{O}_{\text{leaf}}$ in response to the high parameter value in panel 6h), and (d) absence of a mean sensitivity without cancelling effects (e.g. $\delta^{18}\text{O}_{\text{stem}}$, $\delta^{18}\text{O}_{\text{soil}\,7}$ and $\delta^{18}\text{O}_{\text{soil}\,20}$ in panels 6a and 6b).

Although we like the idea of calculating elasticities, in principle, we did see some problems:

- 1) The $\delta^{18}\text{O}$ values are not ratio-scaled (but interval-scaled) and the zero value (0‰) is not an absolute zero, resulting in problems when comparing parameter effects on the $\delta^{18}\text{O}$ of the different water pools.
- 2) ‘Elasticity’ quantifies the percentage change of the output variable in response to a given percent change in the input parameter. This does not consider if a given percent change in the input parameter is hydrologically or physiologically plausible or relevant (particularly when model sensitivity is compared for different parameters).
- 3) It may not be possible to draw universally valid conclusions from the elasticity. In case of a non-linear response of the variable under study, elasticity depends on the extent of change of the parameter. Yet, varying parameters by the same percentages, e.g. by +50% and by -50%, in order to ‘disturb each parameter similarly’, would neglect morpho-physiological or system knowledge on the ‘realistic’ (or ‘plausible’) range of values for each parameter. So, changing a parameter by a certain percentage is likely a more arbitrary choice than the one that we have taken.

Point 3) is also valid for derivatives.

Regarding the second point of the reviewer “one can of course use “arbitrary” ranges of model parameters to look at the output range”:

This is a point that we had discussed extensively, during the work and preparation of the submitted manuscript. In effect, we did not use arbitrary values. Instead, we chose the upper and lower parameter values based on the range of values observed at the site (LAI, canopy height, mesophyll water content), ranges dictated by physical constraints of the system (root distribution), the origin of the $\delta^{18}\text{O}_{\text{rain}}$ data (IsoGSM predictions as opposed to local measurements), or – where we did not have own measurements – based on the range found in the literature for grasses/grassland (ϕ , m_{gs} , g_0 , V_{cmax} and J_{max}). In that way we ascertained realistic and physiologically meaningful upper and lower parameter values in the sensitivity analysis. In a way, this also dictated that we refrain from calculating elasticities.

On the basis of these facts and considerations, we would like to retain the approach to sensitivity analyses presented in the original manuscript. However, we did take the reviewer’s comment/concerns very seriously and improved the presentation and description of the approach. This included: renaming the ‘mean difference’ by ‘mean sensitivity’ (which is more appropriate and illustrative) and standard deviation of the difference by ‘standard deviation of the sensitivity’, and explaining the rationale for the choice of this specific form of sensitivity analysis.

The legend to Fig. 6 now reads:

“Fig. 6: Sensitivity of modelled midday $\delta^{18}\text{O}$ of leaf, stem and soil water at 7 and 20 cm depth to various parameters of the MuSICA model. The sensitivity was tested by varying one parameter while

keeping all other parameters the same as in the standard MuSICA parameter set (Table S1), as detailed in 2.5. Sensitivity (parameter effect) was quantified by two variables: the mean (or average) sensitivity (in %) resulting from the change of a parameter value relative to the reference run, and the standard deviation of the sensitivity which captures the variability of the response to a parameter-change for the different sampling times (displayed by error bars.) Strong averaging (canceling) effects resulting from the change of a parameter value are revealed by large standard deviations of sensitivities. Note that the sensitivity analysis revealed four different combinations of parameter effects: (a) strong mean sensitivities, without cancelling effects, (b) strong mean sensitivities superposed with strong cancelling effects, (c) small mean sensitivities resulting from strong cancelling effects, or (d) absence of sensitivities unrelated to cancelling effects. Parameter identity is given in the upper left corner of each panel. In (a) to (h), blue down-pointing triangles refer to the low parameter value, red up-pointing triangles to the high parameter value of a sensitivity run, based on the range of values observed at the site or – where such values were missing – the range of reported values for grasses or grassland in literature (see Materials and Methods). In (i) the Moldrup submodel for the water vapour effective diffusivity in the soil was replaced by the Penman model. In (j) we used IsoGSM-predicted $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data instead of locally determined $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data for the isoforcing of MuSICA. Note that the low parameter value for Péclet number (a) predicted a far greater deviation of $\delta^{18}\text{O}_{\text{leaf}}$ than any other parameter.

The relevant section of 2.5 was revised accordingly (P9 L21ff):

“Parameter effects (sensitivities) were quantified by two variables: (i) the mean sensitivity relative to the reference run, obtained as the mean differences from the reference run as $(\sum_{i=1}^n (\delta_{\text{sens},i} - \delta_{\text{ref},i})) / n$, with $\delta_{\text{sens},i}$ the $\delta^{18}\text{O}$ of a given water compartment (leaf, stem, or soil at 7 or 20 cm depth) in a sensitivity run and $\delta_{\text{ref},i}$ that in the reference run, for a day i ; and (ii) the standard deviations of the sensitivity, obtained from the differences between $\delta_{\text{sens},i}$ and $\delta_{\text{ref},i}$. The latter illustrated how strongly the effect of a parameter varied between sampling days, and hence how strongly it depended on the conditions encountered on one specific day. Thus, the sensitivity variables (mean and standard deviation of sensitivity) reported if changes in parameter values caused systematic/general effects (shown by the mean sensitivity), or cancelling effects (shown by the standard deviations of the sensitivity), or combinations, or lack of the two.”

Also, paragraph 3.6 and 4.2 were revised for consistency.

R2 7

Lastly, the authors suggest that there is no Péclet effect but rather a second unenriched water pool. While the data seem to support this, I would have expected a much better discussion.

I cannot find any mentioning of the 2D formulation of Farquhar and Gan (2003) while this should probably be the correct model. For example, what would be the effect if the leaf followed exactly this 2D model but the leaves were sampled only partly, not sampling the least enriched part?

We sampled the entire leaf blades and the entire exposed part of the growing leaf blade of grasses, (which was a minor component of the total sample), and trifoliate leaves of white clover. In the case of *Taraxacum officinale*, we included half a leaf blade, severed along the length of the midrib. With that sampling protocol we integrated (but did not resolve) the entire gradients of evaporation-related ^{18}O -enrichment that occurred within the individual leaf blades, permitting (and restricting us to) the use of the whole-leaf version of the ^{18}O -enrichment model used to evaluate the occurrence of a Péclet effect. With that protocol, it was not possible to use the theory presented in the 2D formulation of Farquhar and Gan (2003); hence we used the non-steady-state version of the Péclet model, which is equivalent to that used by Gan, Wong, Yang and Farquhar (2003) for their experimental whole-leaf data.

In the revision we improved the respective paragraph, which now reads (P4 L27ff): “Each leaf sample included all leaf blades, including the exposed part of the growing leaf, but excluding senescent leaves (cf Fig. 1 of Liu et al., 2017) from each of two vegetative tillers of *D. glomerata* and 16 vegetative tillers of *L. perenne*, *P. pratensis* and *P. pratense*, one half of a leaf blade of *T. officinale* (with the latter severed along, but not including, the mid-vein) and two trifoliate leaves of *T. repens*. This

protocol ensured collection of the entire within-leaf evaporative ^{18}O -gradient of all sampled leaf blade tissue of the different species.”

R2 8

The very small discussion starts with the possibility of xylem (or associated tissues) water and non-steady state but then only talks about the latter. I would have loved to see insights about grass blade anatomy, especially from this group who knows it that well.

We did not collect data on the anatomy of sampled leaves, as this was impractical (see also response to R2 9, below).

R2 9

I also do not follow the argument that there is no non-steady-state effect in the missing correlation with transpiration because the model includes non-steady state. The model yes, the data no. Margaret Barbour’s group also claimed to see no Péclet effect but if they plotted their data against the isotopic composition of transpiration rather than xylem, the Péclet effect re-emerged.

Yes, correct, the model included non-steady-state. A significant fraction of the observations originated from non-steady-state conditions, others appeared to be close to steady-state (Figure S9).

In the revision, we looked at the subset of observations that exhibited seemingly near-steady-state ^{18}O -enrichment (about half the data) to verify additionally if the relationship between the proportional difference between observed leaf water ^{18}O -enrichment ($\Delta^{18}\text{O}_{\text{leaf}}$) and evaporative site enrichment ($\Delta^{18}\text{O}_e$) predicted by the Craig-Gordon model ($\Delta^{18}\text{O}_{\text{e,ss}}$) would indicate the existence of a Péclet effect for that subset. Again, we did not observe evidence of such an effect.

In the revision, we deleted the sentence P12 L15-18, replacing it by: “*Also, the relationship between modelled transpiration rate and the proportional difference between the observed $\Delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}$ predicted by the Craig-Gordon model (Fig. S11) was non-significant, revealing no evidence of a Péclet effect. This was also true, when investigating that relationship with a subset of the data that included only the leaves that exhibited near-steady-state ^{18}O -enrichment. This subset was estimated using model output to identify the times when near-steady-state conditions were most likely, and included about half of the data (results not shown).*”

R2 10

The data sampled 7 species while the model describes one mean species. What is the effect of this? Could an averaging of different leaf dynamics not lead to the observed missing correlation with transpiration?

Yes, we also wondered if inability to detect a Péclet effect in the mixed-species leaf sample could have resulted from different leaf water and, hence, ^{18}O -enrichment dynamics in the different species. As we could not answer that question with the data from our grassland ecosystem study, we included ancillary data obtained separately with *Lolium perenne* and *Dactylis glomerata* in different experiments in controlled conditions by Margaret Barbour. These species formed part of the mixed-species sample in our grassland ecosystem. The *L. perenne* data were based on destructive measurements of leaf water ^{18}O -enrichment; conversely, the experiment with *D. glomerata* employed an online gas exchange and equilibrated leaf water method. In both cases, a Péclet effect was not apparent.

In the revision we expanded and improved the discussion of the putative causes for the absence of a Péclet effect or for our inability of detecting one (P15 L23ff):

“*...environmental conditions. We do not know if putative between-species differences in leaf water dynamics and associated ^{18}O -enrichment, or any other morpho-physiological effects e.g. associated with leaf aging, could have led to a missing correlation between the proportional difference between measured leaf water ^{18}O -enrichment and that predicted by the Craig-Gordon model ($1 - \Delta^{18}\text{O}_{\text{leaf}}/\Delta^{18}\text{O}_e$) and transpiration rate. For these reasons, we explored this question with separate studies of *L.**

perenne and D. glomerata, two species that also formed part of the present grazed grassland ecosystem. Again, these studies found no evidence for a Péclet effect, and supported the two-pool model, as there was no relationship between the proportional difference between measured leaf water enrichment and that predicted by the Craig-Gordon model ... ”

R2 11

Overall I compliment the authors on this very nice data set and the very careful modelling, and wish to see the paper published soon.

Thank you!

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The ^{18}O ecohydrology of a grassland ecosystem – predictions and observations

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Abstract. The oxygen isotope composition ($\delta^{18}\text{O}$) of leaf water ($\delta^{18}\text{O}_{\text{leaf}}$) is an important determinant of environmental and physiological information found in biological archives, but the system-scale understanding of the propagation of the $\delta^{18}\text{O}$ of rain through soil and xylem water to $\delta^{18}\text{O}_{\text{leaf}}$ has not been verified for grassland. Here we report a unique and comprehensive

15 dataset of biweekly $\delta^{18}\text{O}$ observations in soil, stem and leaf waters made over seven growing seasons in a temperate, drought-prone, mixed-species grassland. Using [an the ecohydrology part of a physically-based, \$^{18}\text{O}\$ -enabled soil-plant-atmosphere transfer model \(MuSICA\)](#), we evaluated our ability to predict the dynamics of $\delta^{18}\text{O}$ in soil water, the depth of water uptake, and the effects of soil and atmospheric moisture on ^{18}O -enrichment of leaf water ($\Delta^{18}\text{O}_{\text{leaf}}$) in this ecosystem.

20 The model accurately predicted the $\delta^{18}\text{O}$ dynamics of the different ecosystem water pools, [suggesting that the model generated realistic predictions of the vertical distribution of soil water and root water uptake dynamics. Observations and model predictions indicated that Water-water uptake occurred predominantly from shallow \(<20 cm\) soil depths- throughout dry and wet periods in all years, presumably due \(at least in part\) to because of the effects of high grazing pressure on root system turnover and placement.](#) $\Delta^{18}\text{O}_{\text{leaf}}$ responded to both soil and atmospheric moisture [contents](#) and was best described [in terms of constant proportions of unenriched and evaporatively enriched when leaf water was separated into \(two non-mixing water-pool models\)](#). The [else good](#) agreement between model predictions and observations is remarkable [\(and promising\)](#) as model parameters describing the relevant physical features or functional relationships of soil and vegetation were held constant with one single value for the entire mixed-species ecosystem.

1 Introduction

30 The stable oxygen isotope composition ($\delta^{18}\text{O}$) of meteoric water varies greatly in space and time. [During rainfall, meteoric waters imparts its their isotopic signal \(\$\delta^{18}\text{O}_{\text{rain}}\$ \) to that of soil water \(\$\delta^{18}\text{O}_{\text{soil}}\$ \), changing it as a function of refilling, exchange and percolation but this signal \(\$\delta^{18}\text{O}_{\text{rain}}\$ \) is subsequently altered during mixing and other fractionating processes occurring](#)

withinthroughout the soil profile. The oxygen isotope composition of leaf water ($\delta^{18}\text{O}_{\text{leaf}}$) differs from that of the water taken up from the soil, strongly from $\delta^{18}\text{O}_{\text{rain}}$ as leaf water becomes ^{18}O -enriched due to evaporative effects and morpho-physiological controls (Barbour, 2007) undergo an isotopic enrichment during transpiration.

As a consequence, $\delta^{18}\text{O}_{\text{leaf}}$ carries important environmental and physiological information that is imprinted on photosynthetic products and archived in long-lived cellular compounds such as cellulose in tree rings (Farquhar et al., 2007; Barbour, 2007; Treydte et al., 2014; Lehmann et al., 2018). The $\delta^{18}\text{O}$ of leaf water also imprints the oxygen isotope compositions of atmospheric CO_2 and molecular oxygen, a property that can be used to estimate regional and global scale land primary productivity from seasonal to millennium time scales (Dole et al., 1954; Farquhar et al., 1993; Bender et al., 1994; Luz and Barkan, 2011; Wingate et al., 2009; Welp et al., 2011). A quantitative understanding of the hydrological and plant morpho-physiological mechanisms

controlling $\delta^{18}\text{O}_{\text{leaf}}$ is therefore fundamental to biological, Earth and environmental science disciplines (Barbour, 2007). This science, that explores relationships between the spatio-temporal dynamics of water in the soil-vegetation-atmosphere system with help of the dynamics of ^{18}O of water in the different components of the system, may be termed ^{18}O ecohydrology.

Studies that deal with the $\delta^{18}\text{O}$ in water and biomass compartments of grassland, the largest terrestrial biome after forest, are sparse (e.g. Flanagan and Farquhar, 2014; Webb and Longstaffe, 2003, 2006; Ramirez et al., 2009; Riley et al., 2002, 2003).

To our knowledge, simultaneous observations of seasonal variations of the isotopic composition of the different water pools in a temperate grassland ecosystem over multiple years have not been reported so far. Only datasets covering short periods (e.g. Lai et al., 2008; Leng et al., 2013) or one single vegetation period (e.g. Wen et al., 2012) have been reported. In addition, our system-scale understanding of the propagation of the rainwater $\delta^{18}\text{O}$ signal through soil water and plant xylem water to the leaf water has as yet not been verified for grassland. As a consequence, our quantitative knowledge of the drivers of $\delta^{18}\text{O}_{\text{leaf}}$ in grassland ecosystems is limited.

The isotopic composition of the plant source-water taken up by plants ($\delta^{18}\text{O}_{\text{xylem}}$, henceforth equated with termed $\delta^{18}\text{O}_{\text{stem}}$) can vary over time through changes in the depth of soil water uptake by roots or direct changes in soil water isotopic composition. For example, summer rains in continental Europe are usually often-isotopically distinct (^{18}O -enriched) relative to winter precipitation, generating intra-annual variations of $\delta^{18}\text{O}_{\text{soil}}$ ($\delta^{18}\text{O}$ of soil water) with soil depth. Apart from the

temporal distribution of rainfall amounts and associated $\delta^{18}\text{O}_{\text{rain}}$, the relationship between $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{stem}}$ is affected by soil properties (that determine water storage, transport and mixing of rainwater with water stored at depth in the soil profile), the depth distribution of roots and their specific activities and atmospheric conditions and vegetation properties (that determine transpiration, and soil evaporation and associated enrichment of $\delta^{18}\text{O}_{\text{soil}}$ near the soil surface). Assuming that root water uptake proceeds without ^{18}O discrimination (Dawson et al., 2002), the comparison of $\delta^{18}\text{O}_{\text{soil}}$ and $\delta^{18}\text{O}_{\text{stem}}$ can help

identify the depth of root water uptake (e.g. Durand et al., 2007) and how it changes during drought (e.g. Hoekstra et al., 2014; Nippert and Knapp, 2007a). So far, studies on potential shifts of root water uptake depth in C_3 grassland communities during drought were mainly conducted using rainout shelters and comparing the water uptake depth in droughted and control plots (Hoekstra et al., 2014; Prechsl et al., 2015). Thus it is still unclear how edaphic drought arising under natural conditions modifies the root water uptake depth in C_3 grassland communities over time, especially at a multi-seasonal timescale.

The mechanisms driving the isotopic enrichment of leaf water can be studied separately from those driving changes in $\delta^{18}\text{O}_{\text{stem}}$ by expressing all the isotopic compositions of leaf water as enrichments above $\delta^{18}\text{O}_{\text{stem}}$, i.e., $\Delta^{18}\text{O}_{\text{leaf}} = \delta^{18}\text{O}_{\text{leaf}} - \delta^{18}\text{O}_{\text{stem}}$ —if the $\delta^{18}\text{O}$ of water entering the leaf is the same as that taken up by the root system as a whole. The process of evaporative enrichment was first modelled by Craig and Gordon (1965) for open water bodies and adapted to leaves by 5 Dongmann et al. (1974). Many authors have since noted a discrepancy between the ^{18}O enrichment at the evaporative sites predicted by the Craig-Gordon model ($\Delta^{18}\text{O}_{\text{e}}$) and leaf water enrichment ($\Delta^{18}\text{O}_{\text{leaf}}$) (Cernusak et al., 2016). This discrepancy has been interpreted conceptually with two different models called “two-pool” model (Leaney et al., 1985; Yakir et al., 1994) and “Péclet” model (Farquhar and Lloyd, 1993; Farquhar et al., 2007). In the two-pool model, leaf water is assumed 10 compartmentalised between evaporatively ^{18}O -enriched water (supposed to represent mainly mesophyll cells) and un-enriched water (supposed to represent veins and associated ground tissues). In the so-called Péclet model, the mixing of water isotopes within the leaf lamina is assumed incomplete because of a limited back diffusion of heavy water from the evaporative sites to the remaining leaf lamina as a result of the high tortuosity of the path of water within the mesophyll. This incomplete mixing is characterised by a Péclet number p , defined as the ratio of advection to back-diffusion (Farquhar and Lloyd 1993; Cuntz et al., 2007). The two models predict a different effect of transpiration rate on the proportional 15 difference (ϕ) between the ^{18}O enrichment predicted by the Craig-Gordon model and the observed ^{18}O enrichment of leaf water: $\phi = 1 - \Delta^{18}\text{O}_{\text{leaf}}/\Delta^{18}\text{O}_{\text{e}}$ (Song et al., 2013; Cernusak et al., 2016). Because $\Delta^{18}\text{O}_{\text{leaf}}$, rather than $\Delta^{18}\text{O}_{\text{e}}$, imprints sugars 20 (Barbour et al., 2000; Cernusak et al., 2003) and ultimately organic matter (Barbour and Farquhar, 2000; Helliker and Ehleringer, 2002; Barbour, 2007), the choice of the model relating $\Delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}_{\text{e}}$ has important implications. The Péclet model predicts an increase of ϕ with leaf transpiration while in the two-pool model ϕ does not respond to transpiration and is 25 expected to be constant, at least on short (hourly to daily) timescales. Thus far, experimental and empirical studies on a large range of plant species have provided mixed results on these two alternative models of $\Delta^{18}\text{O}_{\text{leaf}}$, with some studies supporting the two-pool model and others the Péclet model (e.g. Barbour et al., 2000, 2004; Loucos et al., 2015; Song et al., 2015; Cernusak et al., 2016). The question as to which model is more appropriate for predicting the $\Delta^{18}\text{O}$ of grassland canopy-scale leaf water is particularly relevant for the modelling of $\Delta^{18}\text{O}_{\text{leaf}}$, and ultimately $\delta^{18}\text{O}_{\text{leaf}}$, at larger temporal and spatial scales. 30 In general, $\Delta^{18}\text{O}_{\text{leaf}}$ responds strongly to changes in atmospheric humidity or the isotope composition of water vapour (e.g. Farquhar et al., 2007) and to changes in stomatal conductance (Wang and Yakir, 1995; Barbour and Farquhar, 2000; Helliker and Ehleringer, 2000; Xiao et al., 2012). However, it is generally not known whether edaphic drought, *via* its effect on stomatal conductance, indirectly affects the relative humidity response of leaf water enrichment. To our knowledge, the only study that reports a distinct effect of edaphic drought on $\Delta^{18}\text{O}_{\text{leaf}}$ is that of Ferrio et al. (2012) on *Vitis vinifera*. Based on their results, and theoretical considerations regarding the effect of soil water availability on leaf stomatal closure and energy budget and associated ^{18}O fractionation, one would expect a positive effect of edaphic drought on leaf water enrichment. Yet, whether or not drought exerts a measurable effect on $\Delta^{18}\text{O}_{\text{leaf}}$ of grasslands, often found in climates with sporadic or prolonged drought periods, is not known.

The interpretation of the isotopic composition of water from samples collected in natural ecosystems is complicated by the fact that multiple environmental, as well as site or plant morpho-physiological factors vary simultaneously, causing difficulties in disentangling the effect of different parameters on the water isotope composition. Hence, process-based ecosystem-scale models are key to aid the interpretation of the water isotope signals in response to environmental and 5 morphological parameters (e.g. Riley et al., 2003). Here we evaluate our system-scale eco-hydrological understanding of the propagation of the $\delta^{18}\text{O}$ signal of rainwater through soil water pools, root water uptake and $\delta^{18}\text{O}$ enrichment of leaf water in a drought-prone grassland ecosystem. For this, we systematically trace, predict and validate $\delta^{18}\text{O}_{\text{soil}}$, $\delta^{18}\text{O}_{\text{stem}}$ and $\Delta^{18}\text{O}_{\text{leaf}}$ and evaluate their sensitivity to input parameters. Specifically, we ask: what is the plant community's depth of root water uptake and does it shift in response to soil water scarcity? Is the two-pool model or the Pécelt model more appropriate for describing 10 $\Delta^{18}\text{O}_{\text{leaf}}$ at the canopy scale? Does $\Delta^{18}\text{O}_{\text{leaf}}$ respond to edaphic drought in grasslands? And more generally: what is the sensitivity of soil, stem and leaf water $\delta^{18}\text{O}$ to changes in soil and vegetation parameters that are suspected to alter ecosystem 15 water dynamics? [To explore these questions we compared predictions from the \$^{18}\text{O}\$ -enabled soil-plant-atmosphere transfer model MuSICA \(Ogée et al., 2003; Wingate et al., 2010; Gangi et al., 2015\) with those observed in a unique, multi-annual data set \(7 years\) of growing season \(April to November\), biweekly samplings and \$\delta^{18}\text{O}\$ analysis of soil water \(at 7 and 20 cm depth\), stem and midday leaf water, atmospheric water vapour, along with rainfall amount and \$\delta^{18}\text{O}_{\text{rain}}\$ data. The experimental site \(Schnyder et al., 2006\) was an intensively grazed *Lolio-Cynosuretum* \(Williams and Varley, 1967; Klapp, 1965\) community with *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, *Taraxacum officinale* and *Trifolium repens* as the main species. Vegetation samples were taken as mixed-species samples, as described below. To explore these questions we compared predictions from the \$^{18}\text{O}\$ -enabled soil-plant-atmosphere model MuSICA \(Ogée et al., 2003; Wingate et al., 2010; Gangi et al., 2015\) with those observed in a unique, multi-annual data set \(7 years\) of growing season, biweekly samplings and \$\delta^{18}\text{O}\$ analysis of soil water \(at two depths\), mixed species stem and midday leaf water, atmospheric water vapour, along with rainfall amount and \$\delta^{18}\text{O}_{\text{rain}}\$ data.](#)

2 Materials and Methods

2.1 Study site

25 The study was performed inside pasture paddock no. 8 of Grünschwaige Grassland Research Station near Freising, Germany (Schnyder et al., 2006). Mean annual air temperature from 2006 to 2012 was 9.3°C, and mean annual precipitation was 743 mm, as measured at Munich airport meteorological station 3 km from the field site. The soil is a Mollic Fluvisol, with a shallow topsoil of low water holding capacity (66 mm plant available field capacity) overlying coarse calcareous gravel. The depth to the groundwater table is around 1.5 m.

30 During the main vegetation period (mid-April to beginning of November) the paddock was grazed continuously by Limousin suckler cows (Schnyder et al., 2006). Animal stocking density was adjusted periodically to maintain a constant sward height

of about 7 cm. This management system aimed at maintaining a constant sward state by continuously balancing pasture grass production and consumption by the grazing cattle.

2.2 Sampling

Precipitation water was collected following events during the vegetation periods of 2007 to 2012, and during winter 5 2007/2008 (see Supplement, Methods S1). Leaf, stem, soil, groundwater and atmospheric moisture samples were collected on non-rainy days, between 11 am and 4 pm CEST (Central European Summer Time). Sampling occurred at approximately biweekly intervals during the vegetation periods from April 2006 to September 2012. Samples were collected at random 10 locations in an area of about 1 ha in the vicinity of an eddy flux tower installed near the centre of the paddock. On each date, two replicate samples of leaf, (pseudo-)stem and soil were collected. Soil samples were taken at two depths (7 and 20 cm) using an auger. Leaf and stem samples were obtained as mixed-species collections of the co-dominant species: four C₃ 15 grasses (*Lolium perenne*, *Poa pratensis*, *Phleum pratense*, *Dactylis glomerata*), one rosette dicot (*Taraxacum officinale*) and one legume (*Trifolium repens*). Each leaf sample ~~was comprised~~included all leaf blades, including the exposed part of the growing leaf, of the integral youngest fully expanded and mature (but ~~not excluding~~ senescing) leaves~~s~~ blades blade tissue, including the exposed part of the growing leaf (cf Fig. 1 of Liu et al., 2017); from each of two vegetative tillers of *D. glomerata* and 20 16 vegetative tillers of *L. perenne*, *P. pratensis* and *P. pratense*, one half of a leaf blade of *T. officinale* (with the latter severed along, but not including, the mid-vein) and two trifoliate leaves of *T. repens*. This protocol ensured collection of the entire within-leaf evaporative ¹⁸O-gradient of all sampled leaf blade tissue of the different species. Stem (xylem) samples comprised the mid-vein of *T. officinale*, the petioles of the two *T. repens* leaves and the basal part of the vegetative grass tillers, except for the outer-most part that was removed as it could have been subject to evaporative enrichment [cf. pseudo-stem in Fig. 1 of Liu et al. (2017)].

Atmospheric moisture was collected by pumping ambient air through a glass coil immersed in a dry ice-ethanol mixture at a flow rate of 1 L min⁻¹ over periods of 2-6 h around noon. Groundwater was sampled from a well located at about 100 m upstream of the ground water flow beneath paddock no. 8.

All plant and soil samples were immediately transferred to 12 mL Exetainer vials (Labco, High Wycombe, UK), sealed and 25 covered with Parafilm. All samples were stored in a freezer at approx. -18°C until water ~~extraction. Water was extract~~ed for two hours using a cryogenic vacuum distillation apparatus with sample vials placed in a water bath with a temperature set to 80 °C (Liu et al., 2016).

2.3 Isotope analysis

Oxygen isotope composition was expressed in per mil (‰) deviation relative to a standard:

$$30 \quad \delta^{18}\text{O} = (R_{\text{sample}}/R_{\text{standard}} - 1), \quad (1)$$

where R_{sample} and R_{standard} are the $^{18}\text{O}/^{16}\text{O}$ ratios of the sample and the V-SMOW standard (Vienna Standard Mean Ocean Water). Samples collected between 2007 and 2012 were analysed by Cavity Ring-Down Spectroscopy using previously described procedures (Liu et al., 2016). Water samples collected in 2006 were analysed with an IsoPrime isotope ratio mass spectrometer interfaced with a multi-flow equilibration unit (both GVI, Manchester, UK). Each sample was measured 5 against a laboratory standard gas, which was previously calibrated against secondary isotope standards (V-SMOW, V-SLAP and V-GISP). Heavy and light laboratory water standards, that spanned the range of $\delta^{18}\text{O}$ values in the dataset, were analysed every five samples. Analytical uncertainty was 0.2‰. $\delta^{18}\text{O}$ measurements obtained by Cavity Ring-Down Spectroscopy were linearly related with those obtained by isotope ratio mass spectrometry ($n = 176$; $R^2 = 0.99$). In a previous study, we found no difference between the results from spectroscopy-based and pyrolysis-based measurements performed using a 10 TC/EA HTC coupled to an isotope ratio mass spectrometer (see Liu et al., 2017).

2.4 MuSICA modelling

The isotope-enabled soil-plant-atmosphere model MuSICA (Ogée et al., 2003; Wingate et al., 2010; Gangi et al., 2015) was parameterised for the studied grassland based on data collected at the site or taken from the literature (for details and parameter values, see below and Supplement, Methods S2 and Table S1).

15 The model was validated with latent energy flux (LE) data obtained from an eddy covariance station (EC) at the site. According to that comparison (Fig. -S1), MuSICA estimates were unbiased ($\text{LE}_{\text{MuSICA}} = 0.997 \cdot \text{LE}_{\text{EC}}$; $R^2 = 0.59$). Further, we compared MuSICA predictions of total plant-available soil water (PAW, mm) in the entire top soil with PAW modelling and data for the same site presented in Schnyder et al. (2006). For the 2007-2012 data, this yielded the relationship $\text{PAW}_{\text{MuSICA}} = 0.99 \cdot \text{PAW}_{\text{Schnyder et al. 2006}} + 7.8$ ($R^2 = 0.83$), and validated using eddy flux data from the same site (Fig. S1).

20 Although the MuSICA model is capable of simulating $\delta^2\text{H}$ of water pools in the soil-plant system, we excluded those data in the manuscript, as (1) we are primarily interested in the processes leading up to the $\delta^{18}\text{O}$ of cellulose, (2) we had noticed discrepancies in the model-data agreement for D/H indicating fractionation (including a surface effect on D/H of soil water at the experimental site; Chen et al., 2016) that are currently not accounted for in the model, and (3) we did not want to overload the paper with extra figures and discussion. Issues of D/H fractionation of water including data from this 25 experimental site will be addressed in a separate paper.

2.4.1 Meteorological forcing and iso-forcing

MuSICA was forced by half-hourly values of meteorological data and $\delta^{18}\text{O}$ of water vapour ($\delta^{18}\text{O}_{\text{vapour}}$) and rainwater ($\delta^{18}\text{O}_{\text{rain}}$). Wind speed, precipitation, air temperature, relative humidity and air pressure data were obtained from the Munich airport meteorological station, located at about 3 km south of the experimental site. Radiation was calculated as the mean of 30 two weather stations located 10 km west and 12 km east of the experimental site. CO_2 concentration was measured at the site by using an open-path infrared $\text{CO}_2/\text{H}_2\text{O}$ gas analyser (LI-7500, LI-Cor, Lincoln, USA). Observations of For $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ observations at the experimental site were used as forcing variables in MuSICA whenever available. If unavailable,

Otherwise, $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ estimates were obtained from globally-gridded reconstructions derived from the isotope-enabled, nudged atmospheric general circulation model IsoGSM (Yoshimura et al., 2011). The [IsoGSM](#) predicted $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ at the grid point relevant to our site were first corrected for their offset with observed data, as predictions were found to be more enriched by 2‰ and 1.3‰ on average compared to the $\delta^{18}\text{O}_{\text{vapour}}$ and $\delta^{18}\text{O}_{\text{rain}}$ measured at the site (Figs. S2–S4).

2.4.2 Soil parameters

Soil structural properties (proportion of quartz and organic matter) as well as hydraulic characteristics (water retention and hydraulic conductivity) were determined on soil core samples taken at the site (Methods S2 and Fig. S5). In MuSICA, the $\delta^{18}\text{O}$ of soil water is predicted based on liquid and vapour phase water isotope transport in the soil column and evaporative enrichment during soil evaporation. MuSICA allows two alternative formulations of the liquid water and water vapour effective diffusivities through the soil matrix. In the first formulation, these effective soil diffusivities increase linearly with the soil volumetric content of the liquid or vapour phase (Penman, 1940) while in the other formulation, proposed by Moldrup et al. (2003), the influence of the pore-size distribution parameter and the total soil porosity is also taken into account. Here, we explore the consequences of using either the Penman or Moldrup soil diffusivity formulation on the prediction of the $\delta^{18}\text{O}$ of soil, xylem and leaf waters.

2.4.3 Canopy and gas exchange parameters

Grassland vegetation at the experimental site was parameterised in terms of canopy structure, the gas exchange properties of leaves, as well as root distribution and hydraulic properties (Table S1). In theory, MuSICA could account for species mixtures and competition for water and light, but this would require parameters for every single species. As the mixed-species samples were dominated by *L. perenne* and *P. pratensis* with closely similar morpho-physiology, we treated the vegetation sample as one plant type, described with one parameter set (Table S1).

The mean leaf area index (LAI; $2.6 \pm 0.7 \text{ m}^2 \text{ m}^{-2}$) and the mean leaf zenithal angle (LZA; $58^\circ \pm 3^\circ$, corresponding to a leaf inclination index (LII) close to zero, typical of a spherical leaf angle distribution) were estimated from compressed sward height measurements made throughout the 2005 to 2012 grazing seasons ($n = 74$ dates with a total of more than 7000 measurements) and calibration functions obtained from parallel measurements of compressed sward height, uncompressed sward height (estimated with a ruler), LAI and leaf zenithal angle (both determined with a LAI-2000, LI-COR, Nebraska, USA) at the site. The vertical distribution of leaf area in the canopy was described based on Wohlfahrt et al. (2003) (Fig. S6). In the standard parameterisation, LAI and LII were set as constants, in agreement with the constant sward state imposed by management practices (see above). In the sensitivity analyses, we also tested the effect of observed variations of sward height, LAI and LII on modelled $\delta^{18}\text{O}$ of the different water compartments.

Leaf turnover is generally high in grassland (Chapman and Lemaire, 1996) including at our experimental site (Schleip et al., 2013). Thus, the co-dominant species (*L. perenne*, *P. pratensis*, *T. officinale* and *T. repens*) had a short and very similar

mean leaf life span of ~460 growing degree days (GDD, with a base temperature of 4°C) throughout the vegetation period (Schleip et al., 2013). As leaf turnover is high, the photosynthetic characteristics of leaves were set constant in the standard parameterisation. Leaf photosynthesis was modelled according to the Farquhar-von Caemmerer-Berry model (Farquhar et al., 1980). Values for the maximum rate of carboxylation (V_{cmax}), the light-saturated potential rate of electron transport (J_{max}) and other photosynthetic parameters were all taken from literature (Table S1). Leaf respiration rate was estimated from measurements made in the dark at the site (Ostler et al., unpublished) and was assumed to be partly inhibited during the day (e.g. Atkin et al., 1997).

Under well-watered conditions, stomatal conductance for water vapour (g_s) was simulated according to the Ball-Woodrow-Berry (BWB) model (Ball et al., 1987). This model has two parameters: m_{gs} , a species-specific non-dimensional parameter that determines the composite sensitivity of g_s to net CO₂ assimilation and to relative humidity and CO₂ concentration at the leaf surface, and g_0 , the basal (or minimal) stomatal conductance. Uncertainties exist regarding the slope parameter m_{gs} and the intercept g_0 (Miner et al., 2017, and references therein). Values for m_{gs} reported by Wohlfahrt et al. (1998) for 13 grassland species from differently managed sites ranged between 6.9 and 24.7, and values for the intercept g_0 (termed g_{min} in their work) ranged between 12 and 193 mmol m⁻² s⁻¹. Likewise, a considerable range of nighttime stomatal conductance (g_{night}) has been reported for C₃ grasses: from 60 to 140 mmol m⁻² s⁻¹ (Ogle et al., 2012; Press et al., 1993; Snyder et al., 2003). Here, g_{night} (together with leaf lamina-water content W , see below) was manually adjusted by fitting MuSICA to diurnal measurements of leaf water δ¹⁸O (Fig. S7). In the standard simulation, we used $m_{\text{gs}} = 10$, a commonly used value for C₃ vegetation (cf. Miner et al., 2017), $g_0 = 10$ mmol m⁻² s⁻¹ and $g_{\text{night}} = 30$ mmol m⁻² s⁻¹. Although the diurnal pattern of δ¹⁸O_{leaf} (Fig. S7) indicated some nocturnal stomatal conductance, the model generally predicted very low nighttime transpiration, in agreement with the eddy flux data (Fig. S1) and the generally high nocturnal relative humidity. Finally, we tested the sensitivity of model predictions to variations of m_{gs} and g_0 (see below).

The effect of edaphic drought on g_s was considered by scaling m_{gs} and g_0 with a function of predawn leaf water potential (Nikolov et al., 1995). This adds two extra model parameters whose values were sourced from the literature (Table S1) and results in a 50% reduction of m_{gs} and g_0 at -1.5 MPa.

Characteristic dimensions of leaves and shoots for the calculation of boundary-layer conductance were estimated based on measurements on individual grass tillers. The width and length (0.1 and 7 cm, respectively) of the leaf blade of a 7 cm-tall grass tiller were taken as minimum and maximum values for the leaf dimensions, and the average leaf dimension was estimated as the square root of the area of such a leaf blade (0.8 cm). Values for minimum, maximum and average shoot dimensions were taken from sward height measurements (see above). The shelter factor was varied between 1 and 3.5 (Monteith and Unsworth, 1990), with very little consequences on the results. Parameter values for leaf optical properties, rain interception and wind attenuation were taken from the literature (Table S1).

In the model, total rooting depth was equated with topsoil depth (37 cm), as in Schnyder et al. (2006). The vertical distribution of fine roots in the soil column was assumed to follow a beta distribution (e.g. Sadri et al., 2018) with a maximum at 7 cm belowground (Fig. S8). The total amount of roots (g m⁻² of soil) was obtained from soil core sampling.

The proportion of live roots was derived from a 14-days long dynamic $^{13}\text{CO}_2/^{12}\text{CO}_2$ labelling experiment at the same site (Gamnitzer et al., 2009; Schleip, 2013; Ostler et al., 2016; Ostler et al., unpublished). Root mass data were converted to root lengths by assuming a specific root length of 100 m g^{-1} (Picon-Cochard et al., 2012). Mean fine root radius was set to 0.15 mm (Picon-Cochard et al., 2012), and root xylem radial hydraulic resistance to $1.0 \cdot 10^{12} \text{ s m}^{-1}$.

5 2.4.4 Oxygen isotope composition of water pools

The steady-state ^{18}O enrichment of leaf water at the evaporative site ($\Delta^{18}\text{O}_{e,ss}$) was calculated [according toas](#) (Farquhar and Lloyd, 1993; Farquhar and Cernusak, 2005):

$$\Delta^{18}\text{O}_{e,ss} = \alpha^+ (\alpha_k (1 - h) + h (\Delta^{18}\text{O}_v + 1)) - 1, \quad (2)$$

where h is the air relative humidity, normalised at leaf temperature (estimated from the leaf energy budget), $\Delta^{18}\text{O}_v$ represents the isotopic composition of atmospheric water vapour, expressed above that of xylem water, α^+ is the isotope fractionation during liquid-vapour equilibrium at leaf temperature (Majoube, 1971) and α_k is the kinetic isotope fractionation during water vapour diffusion through stomata and leaf boundary layer. α_k was estimated at half-hourly time steps from stomatal and boundary-layer conductances for water vapour (g_s and g_b):

$$\alpha_k = 1 + \frac{0.028/g_s + 0.019/g_b}{1/g_s + 1/g_b}, \quad (3)$$

Equation (3) uses the kinetic fractionation factor during molecular diffusion (28‰) reported by Merlivat (1978) and assumes laminar diffusion through the leaf boundary layer (Farquhar et al., 2007).

We modelled leaf water isotope enrichment at isotopic steady state ($\Delta^{18}\text{O}_{leaf,ss}$) using the two approaches introduced earlier. In the “two-pool” simulation, we used a constant value for φ of 0.39, which was chosen such that the observed $\Delta^{18}\text{O}_{leaf}$ was on average predicted without bias. In the sensitivity analysis, φ was varied between -0.20 and 0.50 based on the range of values reported previously for a variety of grass species (Helliker and Ehleringer, 2000; Gan et al., 2003; see Discussion). In the “Péclet” simulation, $\Delta^{18}\text{O}_{leaf,ss}$ was related to $\Delta^{18}\text{O}_{e,ss}$ using the Péclet number, as described by Farquhar and Lloyd (1993):

$$\Delta^{18}\text{O}_{leaf,ss} = \Delta^{18}\text{O}_{e,ss} \frac{1 - e^{-p}}{p}, \quad (4)$$

with p the Péclet number. The latter is calculated as $p = EL/(CD)$ where L (m) is the effective path length, E ($\text{mol m}^{-2} \text{ s}^{-1}$) is the leaf transpiration rate, $C = 55500 \text{ mol m}^{-3}$ is the molar density of liquid water and D ($\text{m}^2 \text{ s}^{-1}$) the diffusivity of H_2^{18}O in liquid water (Farquhar and Lloyd, 1993; Cuntz et al., 2007). In line with the original notion of the Péclet model, one single value of L was applied to the dataset, which was again adjusted such that $\Delta^{18}\text{O}_{leaf}$ was predicted without bias.

Two supplementary experiments were also conducted to directly test the relevance of the Péclet effect in the co-dominant pasture species *L. perenne* and *D. glomerata*. These are described in the Supplement.

As leaf water is not in isotopic steady state for extended periods of the day (Fig. S9), an equation for non-steady state enrichment of leaf water was used in addition to Eq. (2)-(4). Using isotopic mass balance of leaf water and assuming that Eq. (4) holds true also in the non-steady state (Farquhar and Cernusak, 2005), the time evolution of $\Delta^{18}\text{O}_{\text{leaf}}$ was modelled as (see also Farquhar et al., 2007):

$$5 \quad \frac{d(W\Delta^{18}\text{O}_{\text{leaf}})}{dt} = -\frac{E}{a_k a^+ (1-h)} \frac{p}{1-e^p} (\Delta^{18}\text{O}_{\text{leaf}} - \Delta^{18}\text{O}_{\text{leaf,ss}}), \quad (5)$$

where W (mol m⁻²) denotes leaf lamina-water content, expressed on a leaf area basis.

A 27-h time series of community-scale $\delta^{18}\text{O}_{\text{leaf}}$ observed at the site in August 2005 (Fig. S7) was used to fine-tune the parameters controlling leaf water enrichment in MuSICA (mesophyllleaf water content and night-time and minimum stomatal conductance) within the range of values expected for temperate grasslands (for parameter values see Table S1).

10 Because MuSICA predicts different leaf-level variables (e.g. g_s , g_b , h , E , $\Delta^{18}\text{O}_{\text{leaf,ss}}$, ...) for sunlit, shaded, wet or dry leaves at different levels within the canopy, assimilation-weighted canopy averages of $\delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}_{\text{leaf}}$ were first calculated at every time step before performing comparisons with observed data.

2.5 Sensitivity analysis

A sensitivity analysis was conducted in order to quantify the responsiveness of predicted midday $\delta^{18}\text{O}$ of leaf, stem and soil

15 water to plant morpho-physiological parameters that were expected to affect those predictions based on theoretical considerations and/or observed parameter variation at the site. As the leaf water enrichment submodels are embedded in the process-based model MuSICA, the effect of parameters not included in the leaf water $\delta^{18}\text{O}$ models per se could be evaluated.

Based on the *ceteris paribus* principle, the sensitivity was tested by varying one parameter while keeping all other parameters the same as in the standard MuSICA parameter set (Table S1). For a sensitivity run, the parameter was not

20 decoupled from the equations in MuSICA, hence changing one parameter value at the same time affected all equations containing this parameter and all dependent variables. Parameter effects (sensitivities) were quantified by two variables: (1) the mean sensitivity relative to the reference run, obtained as calculating the mean differences from the reference run as

$(\sum_{i=1}^n (\delta_{\text{sens},i} - \delta_{\text{ref},i})) / n$, with $\delta_{\text{sens},i}$ the $\delta^{18}\text{O}$ of a given water compartment (leaf, stem, or soil at 7 or 20 cm depth) in a

sensitivity run and $\delta_{\text{ref},i}$ that in the reference run, for a day i; and (2) Besides, the standard deviations of the sensitivity, obtained from the differences between $\delta_{\text{sens},i}$ and $\delta_{\text{ref},i}$ were calculated for each parameter and water compartment, which The latter illustrated how strongly the effect of that a parameter differs varied between sampling days from day to day, and hence

25 how strongly it depends depended on the instantaneous conditions encountered on one specific day. Thus, the sensitivity variables reported if changes in parameter values caused systematic/general effects (shown by the mean sensitivity), or cancelling effects (shown by the standard deviations of the sensitivity), or combinations, or lack of the two.

30 The high and low parameter values for the sensitivity analyses were chosen according to the range observed for grasses or

grassland species, as reported in the literature or observed at the site (see Supplement). Values for individual parameters of

the sensitivity analysis were set at -0.20 and 0.50 for φ , 1 or 12 mol m⁻² for leaf ~~lamina~~ water ~~volume content~~ (W), 7 or 25 for the slope of the BWB model (m_{gs}), 0 or 193 mmol m⁻² s⁻¹ for the intercept of the BWB model (g_0), 0.6 or 3.8 m² m⁻² for leaf area index (LAI), 3.6 or 11.7 cm for canopy height (h_{canopy}), 20 or 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the maximum rate of carboxylation at 25°C (V_{cmax}) and 32 or 224 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for potential rate of electron transport at 25°C (J_{max}) and 0.08 or 0.265 m for the mean of the vertical root distribution (μ_{root}). V_{cmax} and J_{max} were altered *in tandem* ~~in order~~ to keep the ratio J_{max}/V_{cmax} at 1.6 ([Medlyn et al., 2002](#), [the same as in the standard simulation \(Table S1\)](#)). Apart from those plant morphophysiological parameters, the effect of alternative submodels for the liquid and vapour effective diffusivity in the soil was tested by replacing the Moldrup formulation by the Penman one. In addition, we [investigated the effect of using uncorrected used IsoGSM-predicted \$\delta^{18}\text{O}_{\text{rain}}\$ and \$\delta^{18}\text{O}_{\text{vapour}}\$ data instead of measured local \$\delta^{18}\text{O}_{\text{rain}}\$ and \$\delta^{18}\text{O}_{\text{vapour}}\$ isotopic data \(gap-filled with offset-corrected IsoGSM data; see 2.4.1\)](#) for the isoforcing of MuSICA. [This served in order](#) to illustrate the [usefulness](#) of having local rainwater $\delta^{18}\text{O}$ data.

2.6 Statistics

For comparison of predicted and observed data, we calculated the mean bias error ($\text{MBE} = \bar{P} - \bar{O}$, where \bar{P} is the mean predicted value and \bar{O} the mean observed value) between observed and predicted $\delta^{18}\text{O}$ (or $\Delta^{18}\text{O}$), ~~and~~ the mean absolute error ($\text{MAE} = (\sum_{i=1}^n |P_i - O_i|)/n$), where P_i is the predicted and O_i is the observed value at time i , and n is the number of values; [Willmott and Matsuura, 2005](#)-, [as well as \$R^2\$ values.](#)

[Simple and multiple linear regression](#) ~~Data~~ analyses [and student's t tests](#) were performed in R, version 3.4.2 (R Core Team, 2017) and RStudio, version 1.1.383 (RStudio Team, 2016).

3 Results

20 3.1 Rainfall, $\delta^{18}\text{O}$ of precipitation and vapour

Growing season rainfall amounts and distribution differed between years, with total precipitation in the main growing period (May to August) varying between 321 mm (2006) and 514 mm (2010) (Fig. 1a). The mean $\delta^{18}\text{O}_{\text{rain}}$ signal tended to increase in the first half of the vegetation period and decrease later in the season (Fig. 1b). However, individual rain events sometimes differed markedly from the mean pattern, with excursions of up to +4.5‰ and -6.2‰ relative to the mean of the same month (Fig. 1b). The $\delta^{18}\text{O}_{\text{vapour}}$ signal followed similar mean trends (Fig. 1c), and exhibited a significant correlation ($P < 0.001$) with the $\delta^{18}\text{O}$ of the previous rain event.

3.2 Soil water

~~Volumetric soil water content (SWC) predicted by MuSICA using the standard parameterisation (Table S1) exhibited strong seasonal and inter-annual variations. With SWC values (in m³ m⁻³) expected to vary between 0.19 (permanent wilting point)~~

~~and 0.46 (field capacity), a SWC of less than 0.25 at 7 cm belowground corresponds to <25% of the maximum plant available water at this depth, and is therefore a good indicator of edaphic drought. Each year, soil moisture at 7 cm fell below this threshold, but with a timing that differed from one year to the next (Fig. 1d).~~

The observed $\delta^{18}\text{O}_{\text{soil}}$ was generally more enriched at 7 cm than at 20 cm belowground (Table 1; Fig. 2a, b). This relative enrichment with shallower depth was particularly large in the first half of the vegetation period, and averaged 1.7‰ in the entire data set. The total observed range of $\delta^{18}\text{O}_{\text{soil}}$ differed somewhat between the two depths and was 7.8‰ at 7 cm, i.e., 16% greater than at 20 cm (Table 1).

In most years, $\delta^{18}\text{O}_{\text{soil}}$ followed the rain pattern and increased during the course of the vegetation period at both depths (Fig. 2a, b). This increase was generally more pronounced at 7 cm than at 20 cm. Overall, the seasonal patterns of $\delta^{18}\text{O}_{\text{soil}}$ 10 were quite dynamic, with considerable differences between individual years.

MuSICA simulations with the standard parameterisation (Table S1) predicted the multi-seasonal dynamics of $\delta^{18}\text{O}_{\text{soil}}$ well (Fig. 2a, b) except in 2006 when local data of $\delta^{18}\text{O}_{\text{rain}}$ were not available for the iso-forcing (Fig. 1b) and $\delta^{18}\text{O}_{\text{rain}}$ data were taken from the global atmospheric model IsoGSM, once corrected for the mean model-data offset (Figs. S2–S4). The seasonal trends and monthly fluctuations of observed $\delta^{18}\text{O}_{\text{soil}}$ were reproduced with relatively small error (MAE of 1.1‰ and 15 0.8‰ at 7 and 20 cm, respectively). Also, the bias was small as MuSICA overestimated $\delta^{18}\text{O}_{\text{soil}}$ by 0.8‰ and 0.5‰ at 7 and 20 cm, respectively.

Volumetric soil water content (SWC) predicted by MuSICA using the standard parameterisation (Table S1) exhibited strong seasonal and inter-annual variations. With SWC values (in $\text{m}^3 \text{m}^{-3}$) expected to vary between 0.19 (permanent wilting point) and 0.46 (field capacity), a SWC of less than 0.25 at 7 cm belowground corresponds to <25% of the maximum plant available water at this depth, and is therefore a good indicator of edaphic drought. Each year, soil moisture at 7 cm fell below this threshold, but with a timing that differed from one year to the next (Fig. 1d).

3.3 Stem water

Observed $\delta^{18}\text{O}_{\text{stem}}$ generally matched and followed that of $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm, independently of SWC, season and year (Figs. 2b, c, 3a and S10). Conversely, A similarly close relationship did not exist between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm was generally weak, exhibiting large scatter and a significant offset between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm for most of the data (Fig. 3c). Thus, the MAE (0.7‰) between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm depth was about three times smaller than that for $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm, and only slightly greater than the MAE of 0.5‰ between the replicate samples of soil water taken at 7 cm (Table 2). Remarkably, for 90% of all days on which the soil was classified as 'dry' (predicted SWC<0.25), $\delta^{18}\text{O}_{\text{stem}}$ was still closer to $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm than to $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm, indicating that root uptake did not shift to the lower part of the profile during edaphic drought.

Barnard et al. (2006) showed that the $\delta^{18}\text{O}$ of (pseudo-)stem water in grasses is very close to that of the water taken up by the root systems of grasses (see also Liu et al., 2017), meaning that root water uptake operates without ^{18}O isotope fractionation. MuSICA simulations were based on this assumption and reproduced very similar relationships between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$

as those observed at both depths, with ~~very similar R^2 , MBE and MAE coefficients of determination~~ (Figs. 2-3), ~~thus showing a close agreement between observed and predicted data~~. Importantly, the close correspondence of $\delta^{18}\text{O}_{\text{stem}}$ with $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm depth was not affected by changes in SWC predicted by MuSICA (Fig. 3). Again, the strongest disagreement between predicted and observed $\delta^{18}\text{O}_{\text{stem}}$ occurred in 2006 (Fig. 2c), when observations of local $\delta^{18}\text{O}_{\text{rain}}$ were unavailable.

5 3.4 Leaf water

Midday leaf water $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{leaf}}$) exhibited by far the greatest observed $\delta^{18}\text{O}$ variations in the entire dataset (Table 1). Also, $\delta^{18}\text{O}_{\text{leaf}}$ was unique in the way that it did not exhibit a general trend during the vegetation period ($P = 0.5$; right panel in Fig. 2d). ~~This implied that the observed large temporal variation of $\delta^{18}\text{O}_{\text{leaf}}$ was the result of a short term response. Because, on average, $\delta^{18}\text{O}_{\text{stem}}$ increased over the vegetation period while $\delta^{18}\text{O}_{\text{leaf}}$ did not, $\Delta^{18}\text{O}_{\text{leaf}}$ exhibited a significant decreasing trend over the vegetation period, with a decrease of 0.5‰ per month ($P = 0.01$; right panel in Fig. 2e), in parallel most likely driven by an with increase of the increasing trend of relative humidity over the growing season (data not shown).~~ Conspicuous short-term, parallel increases/anomalies of $\delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}_{\text{leaf}}$ (i.e. changes of $\delta^{18}\text{O}_{\text{leaf}}$ largely independent of variations of $\delta^{18}\text{O}_{\text{stem}}$) occurred occasionally in different years, e.g. in spring of 2008, late spring and early fall of 2009, and early summer of 2010.

15 Predictions of $\Delta^{18}\text{O}_{\text{leaf}}$ with MuSICA agreed best with observations using the two-pool model with $\varphi = 0.39$ ($R^2 = 0.42$; Table 2) in the standard MuSICA parameterisation. This result was robust for different soil water conditions. Unbiased predictions of $\Delta^{18}\text{O}_{\text{leaf}}$ were best obtained by decreasing φ by 0.03 (i.e. setting φ to 0.36) under dry soil conditions ($\text{SWC} < 0.25$) and increasing it by 0.01 (i.e. setting φ to 0.40) under moist soil conditions ($\text{SWC} \geq 0.25$), but this was an insignificant adjustment that did not change the overall coefficient of determination between observed and predicted $\Delta^{18}\text{O}_{\text{leaf}}$.

20 The agreement between observed and predicted $\Delta^{18}\text{O}_{\text{leaf}}$ was always weaker when using the Péclét model. Fixing the effective path length (L) at a certain value led to predictions that were systematically biased for either dry or moist soil conditions (Table 3). Unbiased predictions of $\Delta^{18}\text{O}_{\text{leaf}}$ in conditions of different SWC were only obtained when increasing L (from 0.162 m to 0.235 m) for dry soil conditions and decreasing L for moist soil conditions (from 0.162 m to 0.142 m).

25 MuSICA predictions of $\delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}_{\text{leaf}}$ obtained with the standard parameterisation agreed well with observations at all time scales (Figs. 2d, e, S7 and S9), with low or no bias (MBE of 0.3‰ and 0.0‰, respectively) and an MAE for $\delta^{18}\text{O}_{\text{leaf}}$ of

1.6‰, i.e., 10% of the total variations of $\delta^{18}\text{O}_{\text{leaf}}$ in the entire dataset (Tables 1-2). ~~Also, The the relationship between modelled transpiration rate and the proportional difference between the observed $\Delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}$ predicted by the Craig-Gordon model (Fig. S11) was non-significant, superiority revealing no evidence of a of the two pool model compared to the Péclét model for predicting $\Delta^{18}\text{O}_{\text{leaf}}$ in our dataset was underlined by the absence of a effect relation between transpiration rate and the proportional difference between the observed $\Delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}$ predicted by the Craig-Gordon model (Fig. S11).~~

30 ~~This was also true, when investigating that relationship with a subset of the data that included only the leaves that exhibited near-steady-state ^{18}O -enrichment. This subset was estimated using model output to identify the times when near-steady-state conditions were most likely, and included about half of the data (results not shown). ”~~

3.5 Relationships between soil and atmosphere water status, transpiration, canopy conductance and ^{18}O enrichment of bulk leaf water

Multiple regression analysis demonstrated significant effects of air relative humidity ($P < 0.01$) and SWC ($P < 0.05$) on both observed and predicted $\Delta^{18}\text{O}_{\text{leaf}}$ (Table 4). $\Delta^{18}\text{O}_{\text{leaf}}$ increased with decreasing air relative humidity and SWC (Figs. 4a, b and

5 5a, b). The analysis also indicated a weakly significant interaction effect of air relative humidity and SWC effects was close to significant on for both observed ($P = 0.080$) and predicted ($P = 0.073$) $\Delta^{18}\text{O}_{\text{leaf}}$ (Table 4). Accordingly, the effect of dry soil conditions on $\Delta^{18}\text{O}_{\text{leaf}}$ was most evident at low air humidity (Figs. 4a, b and 5a, b) and was connected with a decrease of canopy conductance (g_{canopy}) (Fig. 5c), estimated here as the ratio of ecosystem scale transpiration rate and air VPD.

10 The modelled dependence of transpiration (estimated with MuSICA) on air VPD (the climatic driver of transpiration) was strongly modified by SWC (Fig. 4c), with h. High air VPD consistently driving drove high transpiration rates only under wet soil conditions ($\text{SWC} \geq 0.25$). Accordingly, the effect of dry soil conditions on $\Delta^{18}\text{O}_{\text{leaf}}$ was most evident at low air humidity (Figs. 4a, b and 5a, b) and was connected with a decrease of canopy conductance (g_{canopy}) (Fig. 5c), estimated here as the ratio of ecosystem scale transpiration rate and air VPD.

3.6 Sensitivity analysis

15 Increasing (decreasing) the proportion of un-enriched leaf water (ϕ) and leaf lamin water volume content (W) led to a strong reduction (increase) in $\delta^{18}\text{O}_{\text{leaf}}$ (Figs. 6a, b). These changes in leaf-level parameters had no effect on $\delta^{18}\text{O}_{\text{soil}}$ or $\delta^{18}\text{O}_{\text{stem}}$. Alterations of stomatal responsiveness (m_{gs}), minimum conductance (g_0), maximum carboxylation (V_{cmax}) or electron transport (J_{max}) rates and LAI had similar directional effects (reflected by the mean sensitivity in relation to the standard simulation) on predicted $\delta^{18}\text{O}$ of soil, stem and leaf water. but However, the strength of the effects differed for the different ecosystem water pools (Fig. 6). Stronger effects were found on $\delta^{18}\text{O}_{\text{leaf}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm, compared to $\delta^{18}\text{O}_{\text{stem}}$ or $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm that tended to vary in close harmony. Generally, a change of the parameter value caused an opposite change of the predicted $\delta^{18}\text{O}$ of a given pool. Moreover, these parameters caused strong cancelling effects, evidenced by large standard deviations of the sensitivity, particularly for $\delta^{18}\text{O}_{\text{leaf}}$. The (unanticipated) sensitivity of $\delta^{18}\text{O}_{\text{soil}}$ to plant morpho-physiological parameters was mediated by related to the effect of those parameters on plant transpiration rate (not shown), which in turn altered the residence time of soil water at the lower depth. For example, lower V_{cmax} and J_{max} values, not accompanied by a change in stomatal responsiveness m_{gs} , led to implied a decrease in transpiration rate and consequent which caused an increase in the percolation of growing season rain water to the lower part of the soil profile (Figs. 7a and 8). In comparison, the ^{18}O -depleted (winter) signal persisted longer in the lower profile at intermediate (Fig. 7b) or high (Fig. 7c) V_{cmax} and J_{max} , as linked higher transpiration rates caused greater drying of the top-soil and reduced during the growing season inhibited the replenishment of deeper soil layers with by summer rainfall.

20 25 30 Apart from LAI, other shoot characteristics, such as canopy height (Fig. 6f), leaf inclination, shoot shelter factor, leaf size and shoot size (not shown) had a very small or no effect on predicted $\delta^{18}\text{O}_{\text{leaf}}$, $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$.

The formulation of the water vapour diffusivity through the soil matrix (Fig. 6i) and the average rooting depth (Fig. 6h) affected $\delta^{18}\text{O}_{\text{soil}}$ (and more strongly so at the lower depth), while the effect on $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{leaf}}$ was much weaker. Not accounting for the pore-size soil particle distribution parameter in the soil diffusivity formulation caused a greater overestimation of $\delta^{18}\text{O}_{\text{soil}}$, especially at 20 cm belowground where the MBE reached 1.3‰, compared to 0.5‰ in the 5 standard run. Shifting the root distribution closer to the soil surface had little effect on $\delta^{18}\text{O}_{\text{soil}}$ at both depths. Conversely, shifting it towards greater depth (Fig. S8) led to an overestimation of $\delta^{18}\text{O}_{\text{soil}}$, especially at 20 cm (Fig. 6h), and increased MAE in the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at both soil depths (not shown).

We also tested the effect of the choice of the water isotope forcing of MuSICA ($\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$). In general, the 10 agreement between predicted and observed ecosystem water pool $\delta^{18}\text{O}$ was much better when MuSICA was forced using locally measured $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data (Fig. 6j). The MBE for the $\delta^{18}\text{O}$ of the different water pools was 3.1 to 6.7-fold greater when using the IsoGSM-based isotope forcing, and the MAE was 1.5 to 2.6-fold higher.

4 Discussion

4.1 Model realism

An isotope-enabled, process-based soil-plant-atmosphere model, MuSICA, generated realistic predictions of multi-seasonal 15 dynamics of $\delta^{18}\text{O}$ in soil, (pseudo-)stem (xylem) and midday leaf water, as well as of the ^{18}O enrichment of leaf water in a drought-prone temperate grassland ecosystem. Throughout the vegetation periods of seven consecutive years (1) model bias (MBE) was low, (2) the range of $\delta^{18}\text{O}$ variations of the different ecosystem water pools was similar in the predictions and 20 observations, and (3) prediction error (MAE) was less than 15% of the total observed range of $\delta^{18}\text{O}$ in the different ecosystem water pools and about twice the size of the MAE for the duplicate samples of the different pools. The relationships between observed $\Delta^{18}\text{O}_{\text{leaf}}$ and variables related to the water cycle such as SWC, air relative humidity, 25 transpiration and canopy conductance were well captured by the model. Although MuSICA is a detailed and locally-parameterised model, this general agreement between model predictions and observations is remarkable given that model parameters describing the relevant physical features or functional relationships of soil and vegetation were held constant with one single value for the entire mixed-species ecosystem. This is a striking outcome given that predicted $\delta^{18}\text{O}$ were found to 30 be quite sensitive to several (but not all) plant morpho-physiological parameters (Fig. 6). The greater scatter in the observed relationship between $\Delta^{18}\text{O}_{\text{leaf}}$ and relative humidity compared to predictions (Fig. 4) probably likely resulted largely partly from sampling effects, in addition to analytical error. Such sampling effects could include small-scale spatial variation of soil properties, or spatio-temporal variation of LAI, nutrient levels and root distribution, a regular feature of grazed grassland (e.g. Schnyder et al., 2006, 2010). Also, Webb and Longstaffe (2003) observed differences of several per mil in 35 $\delta^{18}\text{O}_{\text{soil}}$ in the top 5 cm over distances of about 10 m in a sand dune grassland. Such spatial variations would inherently cause greater scatter in the observations compared to the model predictions.

Prediction of $\delta^{18}\text{O}_{\text{stem}}$ at a given point in time is a real challenge, as $\delta^{18}\text{O}_{\text{stem}}$ is influenced by numerous factors, including the temporal distribution of rainfall amounts and its associated isotopic composition, transport and mixing of rainwater with soil water, the depth distribution of root water uptake in the soil and soil evaporation. These ecohydrological processes are described explicitly in MuSICA, and agreement between observations and predictions of $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm depth indicates that MuSICA is capable of simulating these ecohydrological processes including ^{18}O of the different water pools. The ability of the model to generate realistic predictions of the $\delta^{18}\text{O}$ dynamics at different depths in the soil (within the zone of most active root water uptake and just below that zone) suggests strongly that the ensemble of parameters dictating the spatio-temporal dynamics of soil water contents (including emptying and refilling dynamics) was described well in the model. That interpretation was also supported by the sensitivity analysis. Importantly, a better agreement between predicted and observed $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm and $\delta^{18}\text{O}_{\text{stem}}$ was obtained when the $\delta^{18}\text{O}$ of meteoric water was taken from local measurements rather than given by the isotope-enabled atmospheric model IsoGSM (Fig. 6j). This result is not surprising given the significant spatial and temporal variation of rainfall at weekly and sub-kilometre scales (Fiener and Auerswald, 2009) and the comparatively large grid size of the IsoGSM model simulations (*ca.* 200 km \times 200 km). Our model sensitivity analysis also revealed a better predictive power of the soil diffusivity formulation proposed by Moldrup et al. (2003) over that proposed by Penman (1940) to reproduce the observed isotopic composition of all the ecosystem water pools (Fig. 6i). This superiority was likely related to the effect of accounting for the soil pore size distribution parameter for describing the effective liquid water and water vapour diffusivity through the soil matrix and estimating this parameter from the soil water retention curve parameters measured at the site.

4.2 Xylem water originates from shallow soil depths independently of season and soil water content

The comparison of observed $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ (Fig. 3a) strongly suggested that root water uptake occurred mainly at very shallow depths (<20 cm) throughout the vegetation periods, largely and independently of changes in SWC. This interpretation of observed data was based on comparison of $\delta^{18}\text{O}_{\text{stem}}$ and $\delta^{18}\text{O}_{\text{soil}}$ at two depths (7 and 20 cm) only, which provides limited spatial resolution and cannot inform precisely on the depth of root water uptake (Rothfuss and Javaux, 2017; Brinkmann et al., 2018). Such information can be improved by a locally-parameterised, physically-based, ^{18}O -enabled ecohydrological model, as shown here. For instance, This was well supported by the standard MuSICA runs (Fig. 3b) indicated near-monotonous increases of $\delta^{18}\text{O}_{\text{soil}}$ between 20 and 7 cm depth, matching well the observations in the majority of sampling dates (Fig. S13). Further, the simulations predicted a mean (uptake-weighted) depth of root water uptake above 15 cm in 90% of all sampling dates, independently of SWC and observations of $\delta^{18}\text{O}_{\text{soil}}$ (Figs. S12 and S13). Support came also from the MuSICA and sensitivity analysis (Fig. 6h), showing that $\delta^{18}\text{O}_{\text{stem}}$ was well predicted by the model only when root length density was maximum at shallow soil depths were distributed in very shallow horizons. The potential range of rooting depths is large in grassland, depending on site, species, climatic and management effects (Schenk and Jackson, 2002; Klapp, 1971). So, why would root water uptake be constrained to shallow depths in this drought-prone permanent grassland system? Several factors likely contributed: (1) the shallow top-soil overlying calcareous gravel (Schnyder et al., 2006), (2)

the rapid shoot and root biomass turnover, that is associated with high phytomer dynamics leading to short leaf and root lifespan in intensively managed grassland (Schleip et al., 2013; Yang et al., 1998; Auerswald and Schnyder, 2009; Robin et al., 2010), (3) the high rates of shoot tissue (mainly leaves) losses that elicit a priority for assimilate (including reserve) allocation to shoot regeneration at the expense of the root system (e.g. Bazot et al., 2005), and (4) predominant placement of the root system near the soil surface dictated by the high need for nutrient interception and uptake (e.g. from excreta deposits), to compensate the high rates of nutrient losses due to grazing (Lemaire et al., 2000). Importantly, (5) in a relatively high number of cases, the model predicted situations in which rainfall recharged mainly the top soil, while SWC at depths >20 cm remained low (e.g. June-end of year 2006, April–October 2007, or May-end of year 2008; Fig. S12; see also below). Principally, however, factors (2)–(4) alone can explain why shallow rooting depth is a feature typical feature of intensively grazed grasslands (Troughton, 1957; Klapp, 1971). Also, These results are in line with a recent study of Prechsl et al. (2015) that did not find an increasingly deeper root water uptake upon soil drying in an alpine and a lowland grassland system in Switzerland. Also Similarly, grasses continued to rely on water in the uppermost soil layer during soil water scarcity in a mesic Savanna in South Africa, in which C₄ grasses were growing together with saplings and trees (Kulmatiski and Beard, 2013), and in a tallgrass prairie in the US dominated by C₄ grasses and C₃ shrubs and forbs (Nippert and Knapp, 2007a, b). In the present case, the shallow rooting depth may have been exacerbated by the high grazing pressure and consequent limitations in resource allocation to root growth. Besides, root water uptake of the co-dominant species presumably did not shift to lower horizons due to relatively low water contents in the lower part of the soil profile, as predicted by MuSICA (Fig. S12).

Predictions of $\delta^{18}\text{O}_{\text{soil}}$, particularly below the main zone of most water uptake, at 20 cm, were influenced markedly by estimates of LAI and by changes of V_{cmax} , J_{max} , and stomatal conductance responsiveness (m_{gs}) or minimal value (g_0). This resulted from the effect of those parameters on total canopy transpiration, that in turn altered the dynamics of soil water and hence of the mixing of ¹⁸O-depleted winter and ¹⁸O-enriched summer precipitation with soil water at different depths. For instance, an increase in transpiration rate caused by a high m_{gs} led to a decrease in $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm during the course of the growing season and a growing divergence between observations and predictions, particularly in years with low growing season precipitation (data not shown). This was likely caused by the fact that ¹⁸O-enriched summer rain mainly recharged the upper soil layer in this scenario, as this had been desiccated extensively because of the higher transpiration resulting from the higher m_{gs} . So, summer rains would contribute less to wetting of the lower profile. Conversely, if m_{gs} was set to a low value, predicted $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm increased throughout the vegetation period. According to the same mechanism, the effect of m_{gs} on $\delta^{18}\text{O}_{\text{soil}}$ was negligible when growing season rainfall was high in 2010. The effects of changing V_{cmax} and J_{max} , LAI and minimum conductance on predicted $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm were very similar to m_{gs} , suggesting that these parameters acted via the same mechanism, that is canopy conductance for water vapour that is controlled largely by the (integrated) stomatal conductance of all leaves within the canopy. Thus, the effect of V_{cmax} and J_{max} was likely indirect, resulting from altered assimilation rates impacting stomatal conductance.

4.3 Evidence for a two-pool model of leaf water ^{18}O enrichment

The $\Delta^{18}\text{O}_{\text{leaf}}$ data were well predicted with a two-pool model and a constant fraction of un-enriched water in bulk leaf water ($\varphi \approx 0.39$). This model was valid for a wide range of atmospheric and soil water conditions in seven consecutive growing seasons. Inclusion of a Péclet effect reduced the closeness of fit between measured and modelled $\Delta^{18}\text{O}_{\text{leaf}}$ under all

5 environmental conditions. We did not know if putative between-species differences in leaf water dynamics and associated
 ^{18}O -enrichment, or any other morpho-physiological effects e.g. associated with leaf aging, could have led to a missing
correlation between the proportional difference between measured leaf water ^{18}O -enrichment and that predicted by the Craig-
Gordon model ($1 - \Delta^{18}\text{O}_{\text{leaf}} / \Delta^{18}\text{O}_{\text{e}}$) and transpiration rate. For these reasons, we Supplementary explored this question
studies with separate studies of *L. perenne* and *D. glomerata*, two species that also formed part of the present grazed
10 grassland ecosystem. Again, these studies found no evidence for a Péclet effect, also and supported the two-pool model for
pasture species. as There there was no relationship between the proportional difference between measured leaf water
enrichment and that predicted by the Craig-Gordon model ($1 - \Delta^{18}\text{O}_{\text{leaf}} / \Delta^{18}\text{O}_{\text{e,ss}}$) and transpiration rate in either *L. perenne*
15 plants grown in a controlled environment at different relative humidities and water availabilities, or *D. glomerata* leaves
measured using an online transpiration isotope method (Notes S2 and Figs. S13S14-1415). A two-pool model was also
suggested by the diurnal time courses of $\delta^{18}\text{O}_{\text{leaf}}$ in this grassland (Fig. S7) and in a broadleaf and a coniferous tree species
15 (Bögelein et al., 2017).

When interpreted with the Péclet model, the two-pool model implies a constant Péclet number and inverse variation of transpiration rate and effective path length (L). Dynamic changes of L in response to varying transpiration have been noted before, mainly in controlled conditions, and interpreted in terms of changing contributions of different paths (symplastic, 20 apoplastic, and transcellular) of water movements to the stomatal pore (Barbour and Farquhar, 2003; Kahmen et al., 2008; Song et al., 2013; Loucos et al., 2015; Cernusak et al., 2016). Increases of L in response to drought, as found suggested in this work, have also been observed previously in *Vitis vinifera* by Ferrio et al. (2012), and were connected with variations in leaf lamina hydraulic conductance.

In principle, failure to detect a Péclet effect could be related to the presence of major veins and associated ground tissue of 25 the grass leaves (Holloway-Phillips et al., 2016) or errors associated with non-steady-state effects on ^{18}O enrichment of bulk leaf water (Cernusak et al., 2016). However, MuSICA predictions of $\Delta^{18}\text{O}_{\text{leaf}}$ did account for non-steady state effects and were generally consistent with observed $\Delta^{18}\text{O}_{\text{leaf}}$. The φ value used in our simulations is in the upper range of φ values reported for grasses. Liu et al. (2017) observed species-specific φ values ranging from -0.05 to 0.43 in two C_3 and three C_4 grasses, with no obvious effect of vapour pressure deficit on φ . Gan et al. (2003) presented φ values between *ca.* 0.16 and 30 0.41 in maize, with lower values coming from leaves with the mid-vein removed. Considering a similar effect of vein removal would move our observed φ to about 0.2. Such a value of φ for grasses is very similar to the mean φ reported for a wide range of non-grass species by Cernusak et al. (2016), i.e., close to the mean φ value reported by Cernusak et al. (2016)
for a wide range of (non-grass) species.

4.4 Atmospheric and edaphic effects on the ^{18}O enrichment of leaf water

The strong response of $\Delta^{18}\text{O}_{\text{leaf}}$ to air relative humidity has been observed and discussed previously (e.g. Farquhar et al., 2007; Cernusak et al., 2016), in addition to soil moisture (Ferrio et al., 2012). We are not aware of a previous study that disentangled the separate effects of atmospheric and soil humidity on $\Delta^{18}\text{O}_{\text{leaf}}$, either in field or controlled conditions.

5 Notably, the responses observed in our work were corroborated by theoretical predictions as implemented in MuSICA. Modelled transpiration rate and stomatal conductance were greatly reduced under dry soil conditions, leading to higher kinetic fractionation α_k (Eq. 3) but lower α^+ (Majoube, 1971) and relative humidity h , because of the warmer leaf temperatures. The net effect was a greater $\Delta^{18}\text{O}_{\text{leaf}}$ predicted by MuSICA under dry soil conditions, in agreement with observations. This demonstrated that other vegetation parameters that affected the ^{18}O -enrichment in our sensitivity analysis 10 (e.g. the un-enriched fraction φ or the effective mixing length L , mesophyll-leaf water content W or LAI) but were not considered drought-sensitive, did not seem the main drivers of the enhancement of $\Delta^{18}\text{O}_{\text{leaf}}$ during edaphic drought.

5 Conclusions

This work highlights the usefulness of mechanistic ^{18}O enabled modelling for explorations and quantitative analyses of the hydrology of ecosystems. Such modelling demonstrated here that (1) a single set of plant parameters and site specific soil properties was enough to capture the main $\delta^{18}\text{O}$ dynamics of ecosystem water pools, despite the species mixture characteristic of grassland ecosystems, (2) water uptake occurred from shallow soil depths throughout dry and wet periods in all years, as confirmed by soil and xylem $\delta^{18}\text{O}$ data and model sensitivity analysis on mean rooting depth and (3) $\Delta^{18}\text{O}_{\text{leaf}}$ responded to both soil and atmospheric moisture, and was best described when leaf water was separated into two non-mixing water pools, a result that could be captured solely based on the drought sensitivity of leaf stomatal conductance and photosynthetic capacity, and resulting effects on the leaf energy balance. Demonstration of an effect of soil drying on $\Delta^{18}\text{O}_{\text{leaf}}$ together with reduced stomatal conductance is of great interest for retrospective studies of the functional components controlling water use efficiency of plants. If imprinted on the $\delta^{18}\text{O}$ of plant cellulose, such an effect may also help identify drought events in archived materials, such as the grassland vegetation samples of the Park Grass experiment or herbaria.

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Supplement

The supplement related to this article is available online.

Author contribution

30 JO, RTH and HS designed the study. RTH analysed the data and performed the modelling with guidance by JO. IS and UO designed the sampling scheme and set up, tested the water extraction unit and performed the diurnal water sampling. RS

performed the isotope analysis. SHV analysed the eddy flux data. MMB performed the supplementary controlled environment experiments. RTH and HS wrote the paper. All authors contributed to the discussion and revision.

Competing interests

5 The authors declare that they have no conflict of interest.

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| **Table 1:** Minimum, maximum, mean, and range for the observed $\delta^{18}\text{O}$ of grassland ecosystem water pools (soil water at 20 and 7 cm depth, and stem and bulk leaf water) and ^{18}O -enrichment of leaf water ($\Delta^{18}\text{O}$). Samples were collected at approximately biweekly intervals during the vegetation periods of 2006-2012.

	$\delta^{18}\text{O}$ (‰)			
	Min	Max	Mean	Range
Soil water at 20 cm	-12.3	-5.6	-8.4	6.7
Soil water at 7 cm	-11.3	-3.5	-6.7	7.8
Stem water	-10.4	-3.3	-6.5	7.1
Leaf water	-3.5	12.0	4.1	15.5

	$\Delta^{18}\text{O}$ (‰)			
	4.7	18.2	10.5	13.5

5

Table 2: R^2 , mean bias error (MBE) and mean absolute error (MAE) for the comparison between predicted and observed $\delta^{18}\text{O}_{\text{leaf}}$, $\delta^{18}\text{O}_{\text{stem}}$, and $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm ($\delta^{18}\text{O}_{\text{soil } 7}$) or 20 cm depth ($\delta^{18}\text{O}_{\text{soil } 20}$). Predictions were made with the standard MuSICA parameterisation given in Table S1. Values in parentheses exclude the data from year 2006. The last column presents the MAE between the replicate samples collected on the different dates. MBE and MAE values are given in ‰.

	R^2	MBE	MAE	MAE obs/obs
$\delta^{18}\text{O}_{\text{soil } 20}$	0.79 (0.79)	0.5 (0.6)	0.8 (0.8)	0.6 (0.5)
$\delta^{18}\text{O}_{\text{soil } 7}$	0.56 (0.72)	0.8 (0.5)	1.1 (0.9)	0.5 (0.5)
$\delta^{18}\text{O}_{\text{stem}}$	0.46 (0.60)	0.4 (0.2)	1.1 (0.9)	0.4 (0.4)
$\delta^{18}\text{O}_{\text{leaf}}$	0.43 (0.43)	0.3 (0.2)	1.6 (1.5)	0.8 (0.7)

Table 3: R^2 , mean bias error (MBE) and mean absolute error (MAE) for the comparison between predicted and observed $\Delta^{18}\text{O}_{\text{leaf}}$ obtained with different values of the proportion of unenriched leaf water (φ) in the two-pool model, or effective path lengths (L) in the Pécelt model for the prediction of $\Delta^{18}\text{O}_{\text{leaf}}$. Best predictions are highlighted in bold print. The agreement between predictions and observations was tested for the entire data set ($n = 83$), or the moist (SWC ≥ 0.25 ; $n = 57$) or dry soil subsets (SWC < 0.25 ; $n = 26$). The standard MuSICA parameterisation used a constant $\varphi = 0.39$ for all conditions in all years. MBE and MAE values are given in ‰.

Model	SWC	R^2	MBE	MAE
Two-pool				
$\varphi = 0.36$	all	0.42	0.5	1.5
	moist	0.48	0.7	1.2
	dry	0.38	0.0	2.2
$\varphi = 0.39$	all	0.42	0.0	1.4
	moist	0.48	0.2	1.0
	dry	0.38	-0.6	2.2
$\varphi = 0.40$	all	0.42	-0.3	1.4
	moist	0.48	0.0	1.0
	dry	0.38	-0.8	2.3
Pécelt				
$L = 0.142$ m	all	0.24	0.5	1.9
	moist	0.36	0.0	1.1
	dry	0.12	1.8	3.5
$L = 0.162$ m	all	0.21	0.0	2.0
	moist	0.33	-0.6	1.2
	dry	0.10	1.3	3.6
$L = 0.235$ m	all	0.15	-1.6	2.9
	moist	0.26	-2.3	2.4
	dry	0.05	0.0	3.9

Table 4: Results of a multiple regression analysis of the effects of relative humidity (RH) and soil water content (SWC) on ^{18}O -enrichment of leaf water as observed and as predicted by MuSICA with standard parameterisation. SE, standard error; P , significance level.

Parameter	observed			predicted		
	Estimate	SE	P	Estimate	SE	P
RH	-0.31	0.09	0.001	-0.29	0.06	<0.001
SWC	-41.4	19.2	0.034	-25.2	11.4	0.030
$\text{RH} \times \text{SWC}$	0.59	0.34	0.080	0.36	0.20	0.073
Regression model		R^2			R^2	
		0.44			0.74	

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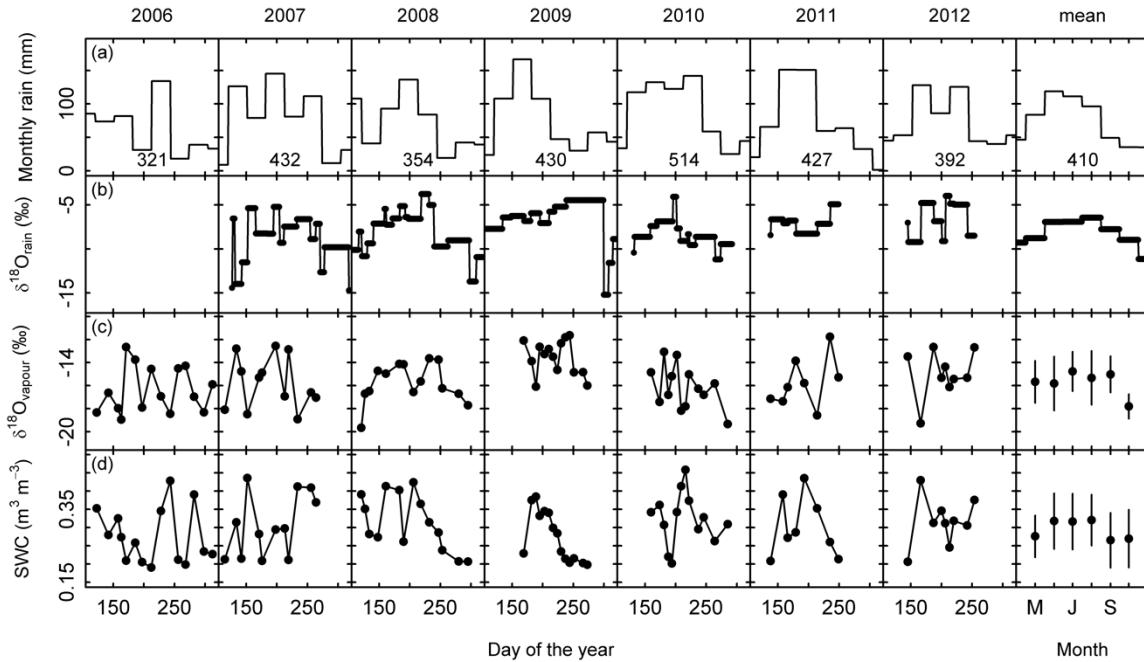
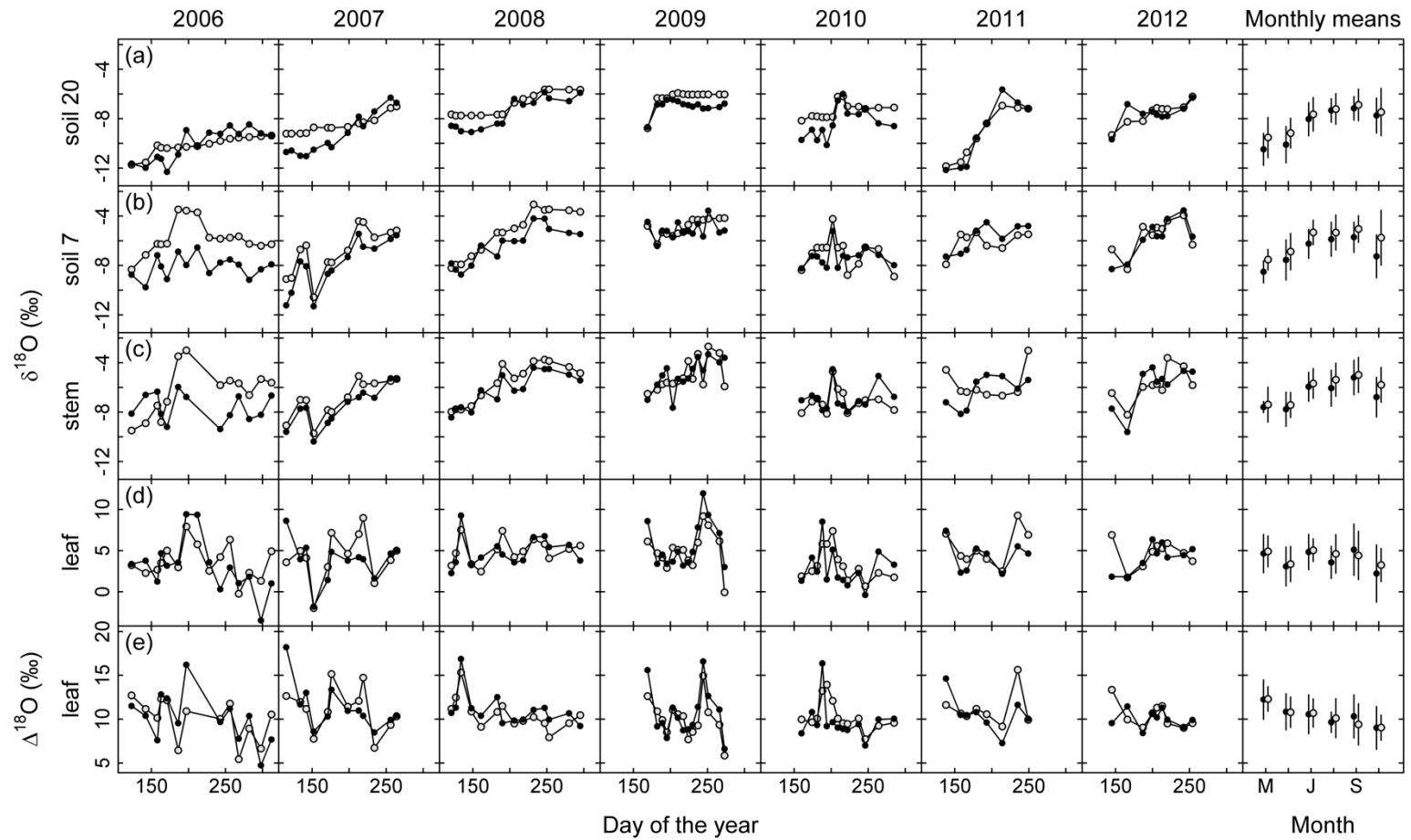


Figure 1: Multi-seasonal (2006-2012) and average patterns of monthly rainfall sums (a), $\delta^{18}\text{O}$ of rain ($\delta^{18}\text{O}_{\text{rain}}$) (b), $\delta^{18}\text{O}$ of atmospheric vapour ($\delta^{18}\text{O}_{\text{vapour}}$) (c), and volumetric soil water content (SWC, m^3 water m^{-3} soil) at 7 cm depth as predicted by the standard MuSICA simulation (d). Permanent wilting point: 0.19 SWC; field capacity: 0.49 SWC. $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ refer to measurements at the experimental site during the vegetation and soil sampling. $\delta^{18}\text{O}_{\text{rain}}$ was determined following individual rains during the vegetation periods of 2007 to 2012. Rainfall data were taken from the DWD weather station of Munich airport, located at the same altitude ~3 km south of the experimental site. The rainfall amount in the main growing period of each year (May to August) is given at the bottom of each panel in (a). Groundwater, at ~1.5 m below the soil surface, had an average $\delta^{18}\text{O}$ of 10.0‰ ($\pm 0.4\text{‰ SD}$).

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5 **Fig. 2: Multi-seasonal (2006-2012) and monthly average variation of $\delta^{18}\text{O}$ in grassland ecosystem water pools: soil water at 20 (a) and 7 cm depth (b), stem (c) and leaf water (d), and ^{18}O enrichment ($\Delta^{18}\text{O}$) of leaf water (e), as observed (closed symbols) or predicted by the standard MuSICA simulations including a two-pool leaf water model (light gray). The parameters for the standard MuSICA simulations are given in the Supplement, Table S1). The error bar in the monthly mean data displays the standard deviation.**

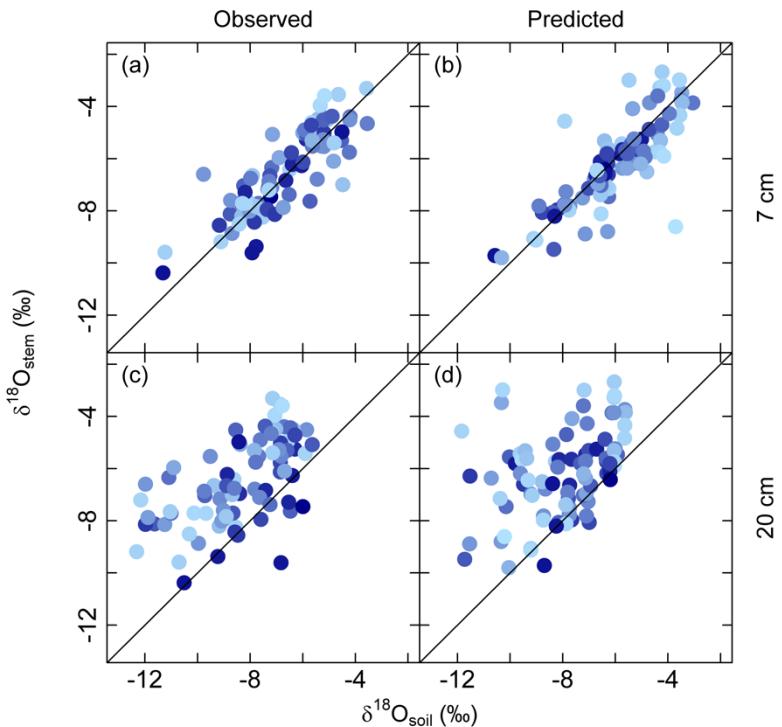


Fig. 3: Correspondence between the $\delta^{18}\text{O}$ of stem water and soil water at 7 cm (observed, (a) and predicted, (b)) and at 20 cm depth (observed, (c) and predicted, (d)). Colour strength indicates soil water content at 7 cm depth as predicted by MuSICA with standard parameterisation: light blue, dry soils; dark blue, soils near field capacity (for colour coding to SWC scale, see Fig. 4). The R^2 , MBE and MAE for the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and the $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm depth were $R^2 = 0.69$, 0.2‰ and 0.7‰ and 0.34 for the observed data (a) comparison and $R^2 = 0.65$, -0.2‰ and 0.7‰ for the predicted data (b). Conversely, the R^2 , MBE and MAE values for the relationship between $\delta^{18}\text{O}_{\text{stem}}$ and the $\delta^{18}\text{O}_{\text{soil}}$ at 20 cm depth and were 0.34, 1.9‰ and 2.1‰ for the observed data (a) and 0.17, 1.8‰ and 1.9‰ for the predicted data (b) for the modelled-modelled relationship. for observed (c) and predicted (d) data. The straight lines represent the 1:1 relationship.

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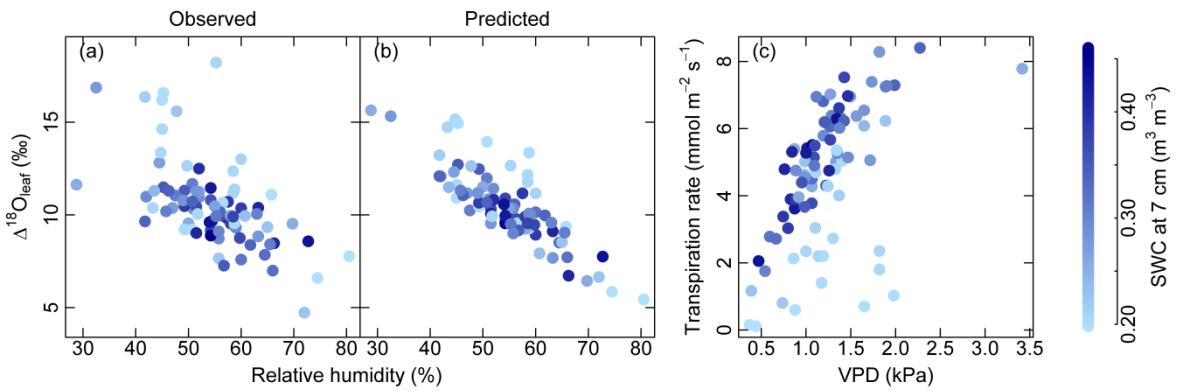


Fig. 4: Relationship between relative humidity of air (RH) and observed $\Delta^{18}\text{O}_{\text{leaf}}$ (a) and predicted $\Delta^{18}\text{O}_{\text{leaf}}$ (b), and modelled response of transpiration to observed vapour pressure deficit (VPD) (c). Strength of blue colour from light to dark indicates the soil water content (SWC) at 7 cm depth as predicted by MuSICA with standard parameterisation. Permanent wilting point: 0.19 SWC; field capacity: 0.49 SWC. Predicted $\Delta^{18}\text{O}_{\text{leaf}}$ data and transpiration rates were obtained with MuSICA in standard parameterisation and a two-pool leaf water model. Multiple regression analysis revealed effects of both RH and SWC on $\Delta^{18}\text{O}_{\text{leaf}}$ (see Table 4).

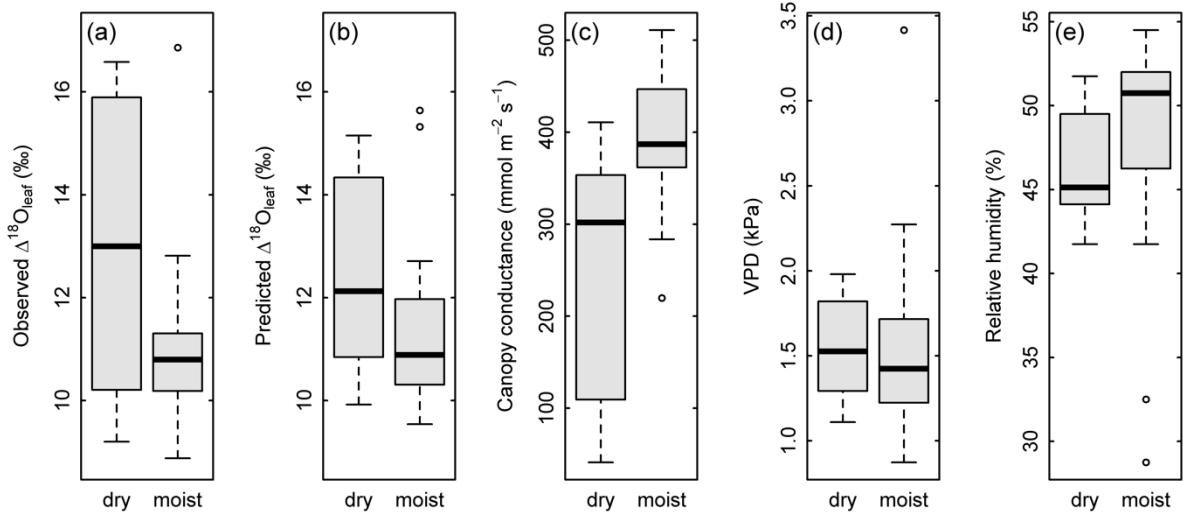


Fig. 5: Boxplots showing the effect of soil water content ('dry' in comparison with 'moist') on observed $\Delta^{18}\text{O}_{\text{leaf}}$ (a), predicted $\Delta^{18}\text{O}_{\text{leaf}}$ (b), and modelled canopy conductance, g_{canopy} (c) under conditions of low air relative humidity (<55% RH). Differences between dry and moist soil conditions were significant at $P=0.03$ (a), 0.06 (b) and 0.003 (c). At the same time, observed air VPD (d) and relative humidity (e) did not differ between dry and moist soil for the displayed subset (RH < 55%). Dry soil was defined as <0.25 SWC ($n = 12$), moist soil as ≥ 0.25 SWC ($n = 29$) at 7 cm depth. With a permanent wilting point of 0.19 SWC and a field capacity of 0.49, a SWC <0.25 corresponded to less than 25% of the maximum plant-available water at 7 cm.

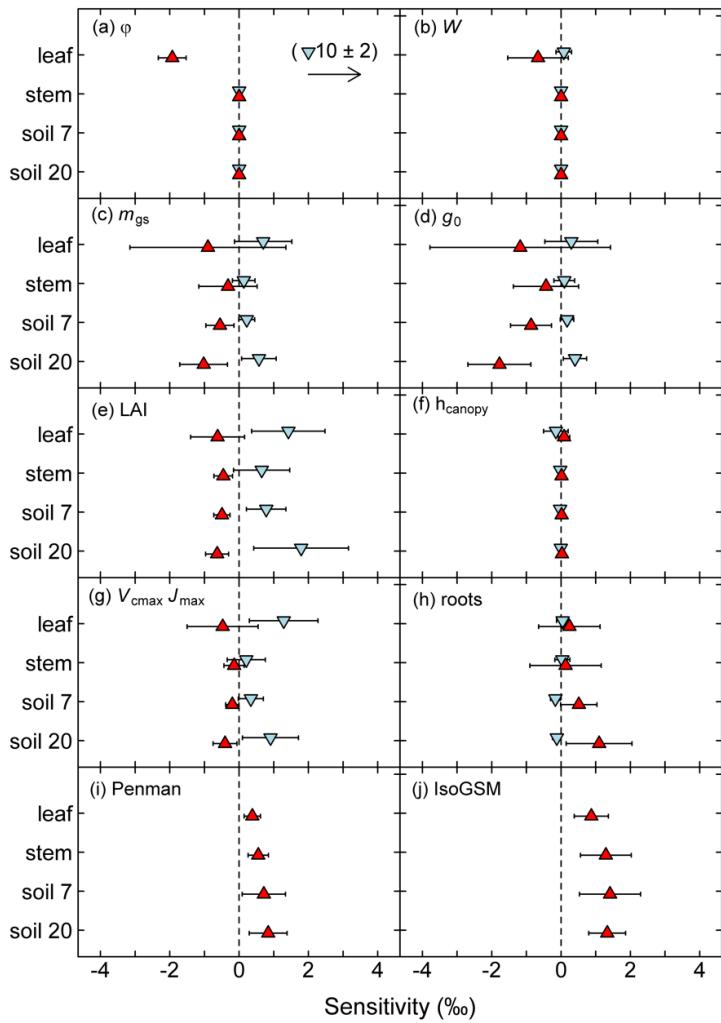


Fig. 6: Sensitivity of modelled midday $\delta^{18}\text{O}$ of leaf, stem and soil water at 7 and 20 cm depth to various parameters of the MuSICA model. The sensitivity was tested by varying one parameter while keeping all other parameters the same as in the standard MuSICA parameter set (Table S1), as detailed in 2.5. and Sensitivity (parameter effect) was quantified by two variables: as the mean (or average) sensitivity (in %) resulting from the change of a parameter value difference from relative to the reference run, and the standard deviation of the sensitivity which captures the variability of the response to a parameter-change for the different sampling times differences, (displayed by error bars (see text). Strong averaging (canceling) effects resulting from the change of a parameter value are revealed by large standard deviations of sensitivities. Note that the sensitivity analysis revealed four different combinations of parameter effects: (a) strong mean sensitivities, without cancelling effects, (b) strong mean sensitivities superposed with strong cancelling effects, (c) small mean sensitivities resulting from strong cancelling effects, or (d) absence of sensitivities unrelated to cancelling effects. Parameter identity is given in the upper left corner of each panel. In (a) to (h), blue down-pointing triangles refer to the low parameter value, red up-pointing triangles to the high parameter value of a sensitivity run, based on the range of values observed at the site or – where such values were missing – the range of reported values for grasses or grassland in literature (see as given in the Materials and Methods). In (i)– the Moldrup submodel for the water vapour effective diffusivity in the soil was replaced– by the Penman model. In (j) we used IsoGSM-predicted $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data instead of locally determined $\delta^{18}\text{O}_{\text{rain}}$ and $\delta^{18}\text{O}_{\text{vapour}}$ data for the isoforcing of MuSICA. Note that the low parameter value for Péclet number (a) predicted a far greater deviation of $\delta^{18}\text{O}_{\text{leaf}}$ than any other parameter.

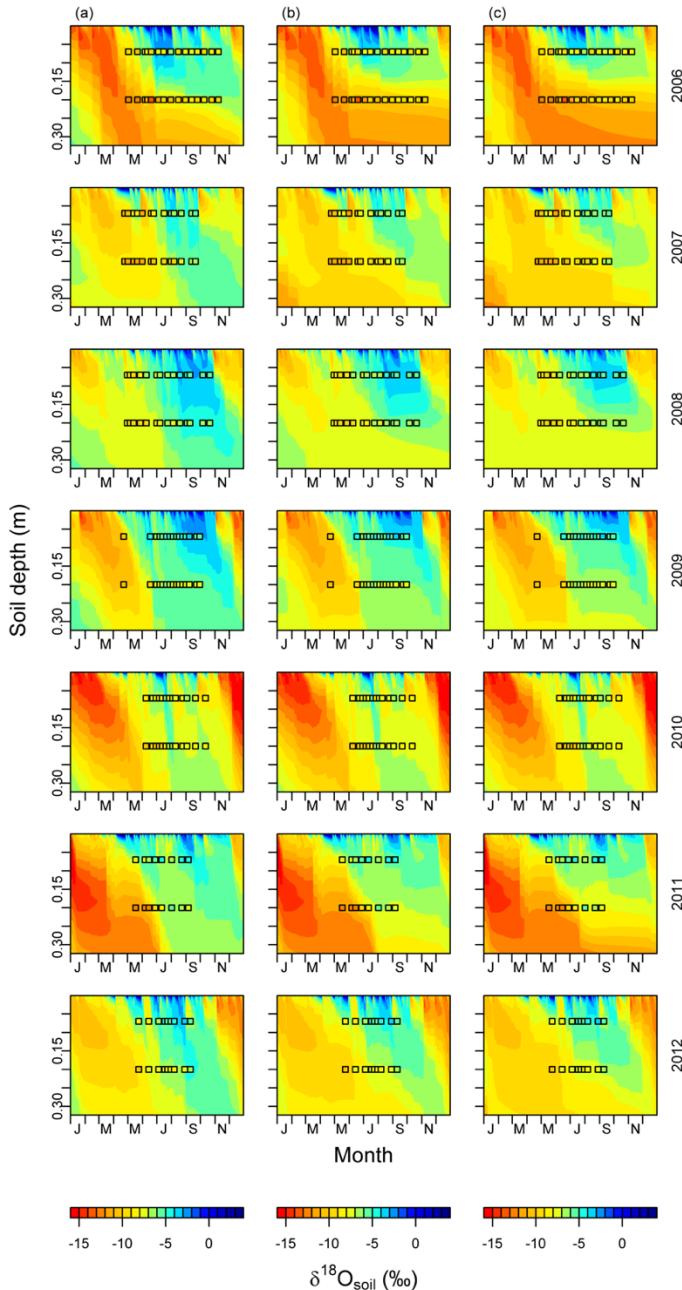


Fig. 7: Soil water $\delta^{18}\text{O}$ dynamics predicted for the studied period (2006-2012) with (a) low, (b) intermediate, and (c) high V_{cmax} and J_{max} . Values for low and high parameter values are given in the Materials and Methods. Observed values for $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm are displayed by squares. The same colour scheme is used for predicted and observed values and for each year and scenario. The abbreviations on the x-axes indicate the months.

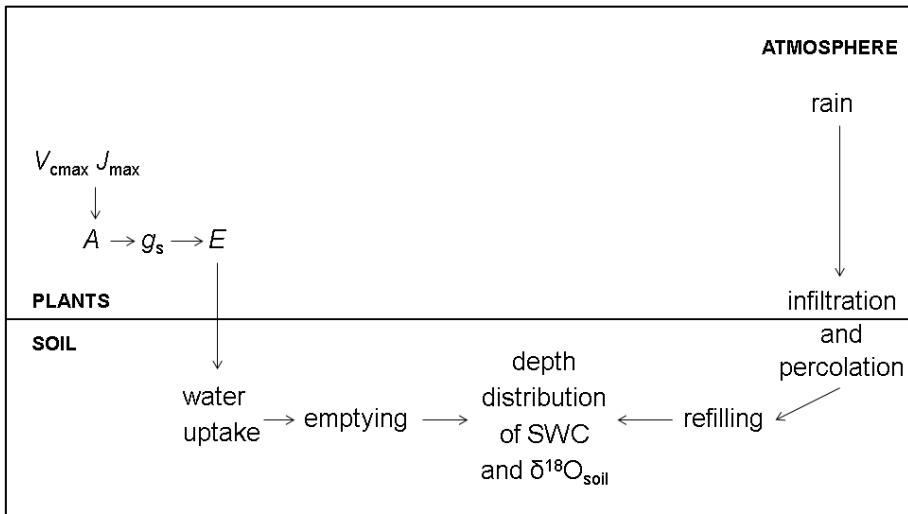


Fig. 8: Flowchart illustrating how changes in photosynthetic parameters (V_{cmax} and J_{max}) affect soil water content (SWC) and isotopic composition ($\delta^{18}\text{O}_{\text{soil}}$).

Methods S1: Precipitation sampling

The sampling apparatus at Grünschwaige pasture paddock no. 8 consisted of a plastic funnel (94 mm in diameter) installed at 1 m above the soil surface and connected to a 1 L plastic collector bottle installed 1 m below ground by means of a silicone hose. A table tennis ball was placed inside the funnel to minimize evaporation losses of collected waters. The bottle was

5 sampled and emptied regularly following rain events, i.e., at intervals of 3 to 61 days (average 14 d; $n = 81$).

Methods S2: MuSICA parameterisation

Parameter values for the ‘standard’ MuSICA runs were derived from data collected at the site (as explained in the main text and below) or taken from the literature (Table S1).

Soil

10 Soil structural properties (proportion of quartz and organic matter) as well as hydraulic characteristics (water retention and hydraulic conductivity) were determined on soil core samples taken at a depth of 3 to 8 cm. Soil water retention and hydraulic conductivity properties were obtained by simultaneously measuring water tension and weight changes resulting from evaporative water loss on 250 mL soil core samples, according to the simplified evaporation method (Schindler, 1980; Peters et al., 2015) using a HYPROP apparatus (UMS, Munich, Germany). Drainage and hydraulic conductivity curves were
15 calculated from water tension and evaporative water loss data using the HYPROP software (Pertassek et al., 2015). Parameters of the van Genuchten-Mualem soil water retention model (van Genuchten, 1980; Mualem, 1976) and of the Brooks-Corey hydraulic conductivity model (Brooks and Corey, 1964), both used in MuSICA, were obtained by least-squares fit to the drainage and conductivity curves (Fig. S5). Gravitational water flow was assumed at the bottom of the mineral topsoil, at 37 cm belowground. Estimated parameter values for the soil surface resistance to water vapour transport,
20 soil surface aerodynamic resistance and soil optical properties (albedo and emissivity) were taken from the literature (Table S1).

In the Moldrup et al. (2003) model for the water vapour effective diffusivity, the pore-size distribution parameter b was derived from the water retention curve parameters m and n as $b = 1/m/n$. In this work, we explore the consequences of using either the Penman or Moldrup soil diffusivity formulations on the prediction of the $\delta^{18}\text{O}$ signals of soil, xylem and leaf
25 waters (see sensitivity analysis in main text).

Soil respiration (the total of root and heterotrophic soil respiration) was predicted using a Q_{10} relationship with soil surface temperature, with basal soil respiration rate at 25°C (R_{25}) and the Q_{10} value obtained from open-top chamber respiration measurements performed at the site in September 2006, May 2007 and September 2007 (Gamnitzer et al., 2009; Ostler et al., unpublished).

Notes S1: Diel measurements and modelling of ^{18}O enrichment of pasture vegetation

Leaf and soil water, and atmospheric moisture were sampled at intervals between 4 am on 4 August to 7 am on 5 August in 2005, in the centre of pasture paddock no. 8 at Grünschwaige. The procedures followed the same protocols as given in the Materials and Methods of the main text, except that soil water was collected at depths of 2, 12 and 22 cm. Leaf samples were 5 collected every hour with three replicates, soil samples every six hours with five replicates at 2 cm, three replicates at 12 cm and one replicate at 22 cm depth.

Fig. S7 shows the diurnal cycle of observed ^{18}O enrichment of leaf water above soil water ($\Delta^{18}\text{O}_{\text{leaf}} = \delta^{18}\text{O}_{\text{leaf}} - \delta^{18}\text{O}_{\text{soil 7}}$), and of the $\Delta^{18}\text{O}$ predicted in the standard simulation (two-pool model with $\varphi = 0.39$) and in the Péclet simulation with $L = 167$ mm. Observed $\Delta^{18}\text{O}_{\text{leaf}}$ reached its minimum (1.9‰) at around 5 am (UTC) – pre-dawn – and then increased 10 progressively for about 5 h to approach a near-maximum value at around 10 am. The observed $\Delta^{18}\text{O}_{\text{leaf}}$ remained within 90% of maximum for about 5 h and then decreased continuously for about 12 h to reach another minimum (at ~0.1‰) at 2 to 5 am the next morning.

These $\Delta^{18}\text{O}_{\text{leaf}}$ data were used to fine-tune the parameters controlling leaf water enrichment in MuSICA, mainly 15 [mesophy4leaf](#) water content, the Péclet effective length and stomatal conductance parameters such as nighttime and residual stomatal conductance, within the known range for temperate grassland or cool-season grasses. Following these adjustments, modelled $\Delta^{18}\text{O}_{\text{leaf}}$ followed quite closely the temporal pattern of observed $\Delta^{18}\text{O}_{\text{leaf}}$ when a two-pool model was applied. In particular, the maximum of modelled $\Delta^{18}\text{O}_{\text{leaf}}$ was reached at approximately the same time as that observed. By contrast, when a Péclet model with a constant mixing length was applied in the simulation, predicted $\Delta^{18}\text{O}_{\text{leaf}}$ reached a maximum in the late afternoon and evening hours that was not present in the observed data (Fig. S7).

20 Notes S2: Testing the relevance of the Péclet effect in the pasture species *Lolium perenne* and *Dactylis glomerata* in controlled environments

Several recent studies (Roden et al., 2015; Song et al., 2015) have called into question the relevance of the Péclet effect to 25 leaf water isotopes. Given this uncertainty, and the added complexity of including a Péclet effect in leaf water models, we tested the requirement for a Péclet effect in the pasture grasses *L. perenne* and *D. glomerata* – two of the co-dominant species in the grassland ecosystem study – with an aim to applying Occam's razor principle if appropriate (Figs. S12-13).

Lolium perenne

Perennial ryegrass seeds (*L. perenne* L. cv. Bronsyn plus AR1 endophyte, 2 g per pot or 83 g m⁻²) were sown into 5-L pots containing 1700 g of seed-raising mix at field capacity and grown in a controlled-environment growth cabinet maintained at 20°C, 70% RH, 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR during the 16-h light period, and 15°C, 70% RH during the 8-h dark period, for 17 d.

30 The pots were then randomly allocated to either high (70%) or low (30%) relative humidity cabinets in which all other settings were the same. All plants were clipped to 6 cm in height, and well-watered daily. Seven days after the humidity

treatments were applied, eight pots within each humidity treatment were allocated to either well-watered (field capacity) or droughted (midway between field capacity and oven-dried water content) treatments. Plants in these pots were again clipped to 6 cm in height. Water content was maintained in both treatments by daily gravimetric measurements, with water used replaced. Plants were grown for 21 days after the commencement of the water treatment and droughted pots took 2-3 days to 5 reach their target water content.

Leaf gas exchange measurements occurred between 8 and 16 days after the start of the water treatment, and leaf water sampling on day 20 of the treatment. Transpiration rate (E) was measured on a group of 10-20 leaves in each of 5 pots per treatment over a 24 hour period under growth conditions using a custom clear-top chamber fitted to a Li6400 (LiCor Inc., Lincoln, NE, USA) photosynthesis system (as described in Loucos et al., 2015, except that the incident light within the 10 growth cabinet was used rather than an external light source). Measurements were recorded every minute, averaged over 10 minutes, then a treatment average calculated to compare to leaf samples taken from randomly-assigned pots every two hours. Every 2 hours when the cabinet lights were on during a 29 hour period, three leaves (3 cm in length) were cut and immediately placed in small glass vials, then flushed with 2% CO₂ and sealed. The oxygen in leaf water was left to equilibrate with oxygen in CO₂ within the vial for 48 hours at 25°C, then the CO₂ was analysed for $\delta^{18}\text{O}$ on a tunable diode 15 laser absorption spectrometer (TDL, TGA100A, Campbell Scientific) as described by Song and Barbour (2016), with liquid water standards for correct isotope compositions of the leaf water relative to SMOW.

The isotope composition of water vapour and irrigation water was measured on the TDL as described above. Water vapour was collected by pumping air from each growth cabinet through a glass cold finger trap sitting in an ethanol-dry ice slurry. Air was pumped for 20 minutes for the low RH cabinet and 10-25 minutes for the high RH cabinet, and collections were 20 made every 2 hours. The irrigation water had a $\delta^{18}\text{O}$ of -9.6‰, while the water vapour varied between -18.2 and -14.0‰ (the low RH cabinet had significantly less enriched water vapour than did the high RH; $-16.0 \pm 0.4\text{‰}$ compared to $-17.2 \pm 0.3\text{‰}$, $P = 0.003$). Irrigation water and vapour $\delta^{18}\text{O}$ were used to calculate $\Delta^{18}\text{O}_{\text{e,ss}}$ (using Eq. (2), main text) and measured leaf water enrichment, $\Delta^{18}\text{O}_{\text{leaf}}$.

The Péclet effect predicts a positive relationship between E and the proportional difference between $\Delta^{18}\text{O}_{\text{leaf}}$ and $\Delta^{18}\text{O}_{\text{e}}$, but it 25 can be seen from Figure S12 that variation in E explained very little variation in the proportional difference, suggesting that the Péclet effect was of limited relevance for *L. perenne*.

Dactylis glomerata

We also tested the relevance of the Péclet effect on a second, small stature grass species using the online gas exchange and equilibrated leaf water method developed by Song et al. (2015). *D. glomerata* L. plants were grown from seed in 7-L pots 30 with potting mix amended with slow release fertiliser (Osmocote, Scotts Australia Pty Ltd., Sydney, NSW, Australia) in a controlled environment room set at day/night temperature of 28/20 °C, 75% air humidity in the day and night, 14 h day period and an approximate irradiance at the top of the canopy of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. When the plants were 60 days old, 3-5 leaves were sealed in a 2 × 3 cm leaf chamber with a red-blue light source attached to a Li6400 photosynthesis system and

plumbed to a water vapour isotope analyser (L1102-i; Picarro Inc., Sunnyvale, CA, USA) for isotopologue measurement. Dry air entered the leaf chamber, so that all the water vapour measured by the analyser came from transpiration (E). The conditions inside the leaf chamber were manipulated to achieve a range in E , by altering flow rate through the chamber (between 250 and 700 $\mu\text{mol s}^{-1}$) and CO_2 concentration (between 100 and 500 $\mu\text{mol mol}^{-1}$), while temperature and irradiance 5 were held constant (30°C and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). Leaves remained in the chamber for 15–20 minutes, after which they were rapidly sampled into glass vials, flushed with 2% CO_2 and sealed prior to equilibration and subsequent isotope analysis as described above (following Song and Barbour, 2016).

There was no significant relationship between E and the proportional difference in *D. glomerata* using the online transpiration technique, consistent with the observation in *L. perenne* (Fig. S13).

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Table S1: Soil and plant parameters used in the standard MuSICA simulations.

Parameter	Symbol	Value	Unit	Comment ^A
SOIL				
Structural characteristics				
Depth	d_{soil}	0.37	m	measured
Quartz fraction	f_{quartz}	0.16	% (w/w)	measured
Organic fraction	f_{organic}	0.07	% (w/w)	measured
Remaining soil fraction	$f_{\text{remaining}}$	0.77	% (w/w)	measured
Bulk density	D_b	1.33	g cm ⁻³	measured
Hydraulic characteristics				
Saturated water content	θ_{sat}	0.49	m ³ m ⁻³	calculated from water retention curve
Residual water content	θ_{sat}	0.01	m ³ m ⁻³	calculated from water retention curve
Retention curve inflection point	α^{-1}	0.43	m	calculated from water retention curve
Retention curve shape factor	m_{ret}	0.13	-	calculated from water retention curve
Hydraulic conductivity at saturation	K_{sat}	0.29	m d ⁻¹	calculated from hydraulic conductivity measurement
Preferential flow				
Fraction of cracks	f_{crack}	0.02	-	estimated
Depth of cracks	d_{crack}	0.1	m	estimated
Resistance to water transport through soil surface pores				
Minimum resistance	$r_{s,\text{min}}$	800	s m ⁻¹	Kelliher et al. (1986)
Maximum resistance	$r_{s,\text{max}}$	16100	s m ⁻¹	Kelliher et al. (1986)
Threshold water content	θ_{tr}	0.194	m ³ m ⁻³	Schaap and Bouting (1997), Ogée and Brunet (2002)
Soil and root respiration				
Respiration at 25 °C	R_{25}	8.5	µmol m ⁻² s ⁻¹	Gamnitzer et al. (2009), Ostler et al. (unpublished)
Base for exponential soil respiration eqn.	Q_{10}	2.2	-	
Surface optical properties				
Surface albedo (of litter or mosses) for visible light	α_{vis}	0.15	-	Deardorff (1978)
Surface albedo (of litter or mosses) for near-infrared light	α_{nir}	0.60	-	--
Surface emissivity	$\varepsilon_{\text{soil}}$	0.95	-	Deardorff (1978)
Soil surface aerodynamic resistance				
Aerodynamic coefficient	C_u	33	-	Ogée and Brunet (2002)
VEGETATION				
Canopy structure				
Canopy height	h_{canopy}	0.078	m	estimated from sward height measurements
Leaf area index	LAI	2.6		estimated from sward height measurements
Mean relative height of vertical leaf area	μ_b	0.315	-	based on Wohlfahrt et al. (2003)

density profile				
Standard deviation of vertical leaf area density profile	σ_b	0.21	-	based on Wohlfahrt et al. (2003)
Leaf inclination index				
	LII	0	-	estimated from sward height measurements
Leaf photosynthesis				
Maximum rate of carboxylation at 25°C	V_{cmax}	60	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Rogers et al. (1998)
Potential rate of electron transport at 25 °C	J_{max}	100	$\mu\text{mol m}^{-2} \text{s}^{-1}$	calculated from V_{cmax} following Medlyn et al. (2002)
Temperature optimum for V_{cmax}	$T_{opt,V}$	40	°C	Harley et al. (1992)
Temperature optimum for J_{max}	$T_{opt,J}$	35	°C	Harley et al. (1992)
Curvature of J -PAR relationship	θ_J	0.85	-	-
Efficiency of light energy conversion (electrons per photon)	α_J	0.18	mol mol^{-1}	Wullschleger (1993) and papers cited therein
Dark respiration rate at 25 °C	R_d	0.86	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Ostler et al. (unpublished)
Light inhibition factor for R_d	I	0.5	-	cf. Atkin et al. (1997)
Stomatal conductance				
Intercept	g_o	10	$\text{mmol m}^{-2} \text{s}^{-1}$	Collatz et al. (1991)
Slope	m_{gs}	10	-	Miner et al. (2017), and references therein
Critical water potential	Ψ_{gs50}	-1.5	MPa	Braud et al. (1995)
Steepness parameter	v	4	-	Nikolov et al. (1995)
Minimum conductance for dawn and dusk conditions	g_{min}	10	$\text{mmol m}^{-2} \text{s}^{-1}$	-
Maximum nocturnal conductance	g_{night}	30	$\text{mmol m}^{-2} \text{s}^{-1}$	fitted (see SI text)
VPD threshold for nocturnal conductance	VPD_{thresh}	0.10	MPa	-
Mesophyll conductance				
Maximum mesophyll conductance	g_m	0.35	$\text{mol m}^{-2} \text{s}^{-1}$	Warren (2008)
Leaf boundary-layer conductance				
Leaf size	d	8	mm	measured and estimated (see SI text)
Shoot size	d_s	78	mm	calculated from sward height measurements
Shelter factor	P_d	1.3	-	Monteith and Unsworth, 1990
Root distribution				
Mean of the β -distribution	μ_{root}	0.105		estimated
Standard deviation of the β -distribution	σ_{root}	0.06		estimated
Mean root length density		19	km m^{-2}	estimated (see Materials and Methods)
Root hydraulics				
Fine root radius	r	0.15	mm	Picon-Cochard et al. (2012)
Root hydraulic resistance	R_{root}	1	Ts m^{-1}	estimated
Total internal storage capacity	W_{cap}	0.01	$\text{kg m}^{-2} \text{MPa}^{-1}$	estimated
Leaf optical properties				

Reflectance for visible light	ρ_{vis}	0.105	-	Sellers (1985)
Reflectance for near-infrared light	ρ_{nir}	0.577	-	Sellers (1985)
Transmittance for visible light	τ_{vis}	0.07	-	Sellers (1985)
Transmittance for near-infrared light	τ_{nir}	0.248	-	Sellers (1985)
Leaf emissivity	$\varepsilon_{\text{leaf}}$	0.98	-	Nikolov et al. (1995); Braud et al. (1995); Jackson (1988)
Rain interception				
Water storage capacity	S	0.1	mm m^{-2}	Deardorff (1978); Braud et al. (1995)
Exponent for power function		0.67		
Wind attenuation				
Canopy drag coefficient	C_d	0.2	-	Massman and Weil (1999)
Leaf water isotope modelling				
<u>Mesophyll-Leaf</u> water content	$W_{\text{mesophyll}}$	2	mol m^{-2}	fitted (see SI text)
Proportion of unenriched leaf water	φ	0.39	-	this work
Peclet effective length	L	0.162	m	this work

^A For details of parameter estimation or measurements, see Materials and Methods in main text and Supplemental Information

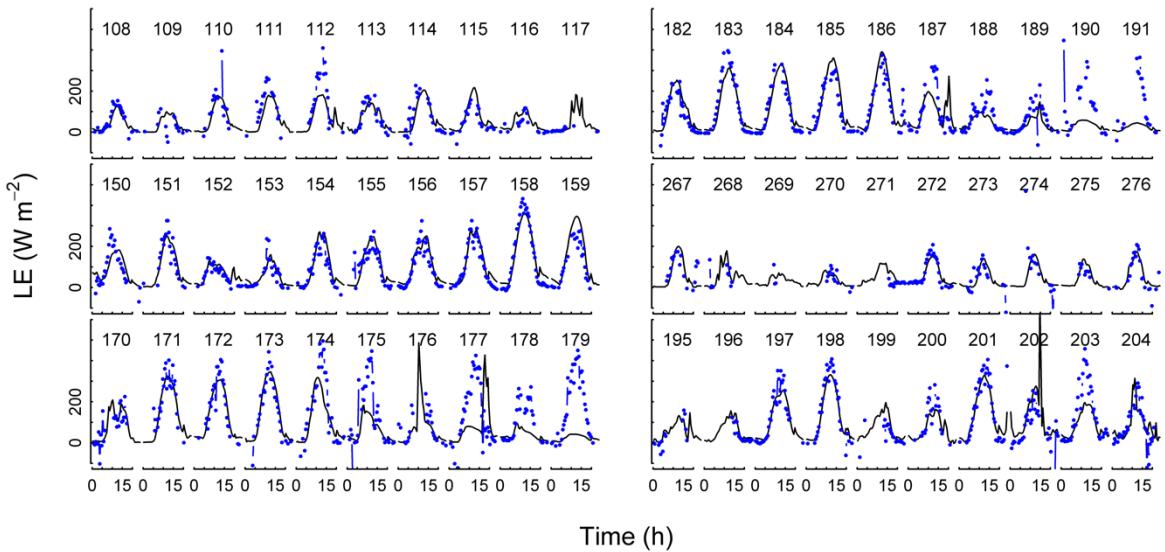


Figure S1: Comparison of latent heat flux obtained from eddy flux data (blue dots) and latent heat flux predicted by the MuSICA model in standard parameterisation (continuous black line). Panels show 10 d-long periods selected randomly from the first (left panels) and second half (right) of the vegetation periods of 2006 (top) to 2008 (bottom). The numbers above the diurnals indicate the day of the year. Time is given in UTC. Both data sets were obtained at pasture paddock no. 8 of Grünschwaige Grassland Research Station. [The relationship between eddy flux and MuSICA-based estimates of latent heat fluxes for the entire 2006–2008 data set was not biased and had an \$R^2 = 0.60\$.](#)

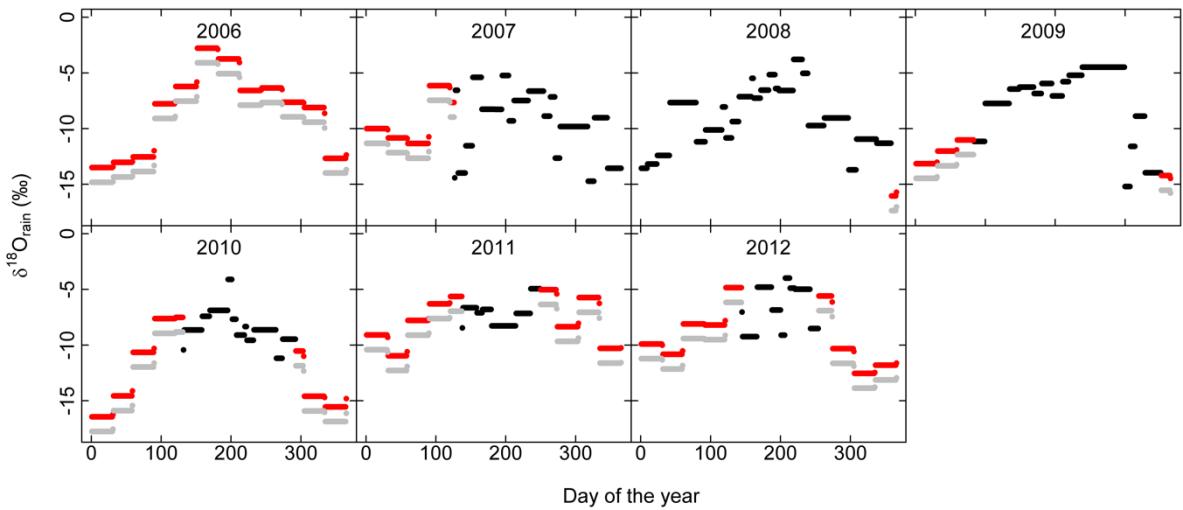


Figure S2: $\delta^{18}\text{O}$ of rain water ($\delta^{18}\text{O}_{\text{rain}}$) collected at the experimental site (black symbols), along with IsoGSM predictions (red symbols) and corrected IsoGSM predictions of $\delta^{18}\text{O}_{\text{rain}}$ (grey symbols). The latter were obtained by subtracting the mean offset ($-1.3\text{\textperthousand}$; cf Fig. S3) between $\delta^{18}\text{O}_{\text{rain}}$ observed at the site and IsoGSM predictions from the non-corrected IsoGSM data.

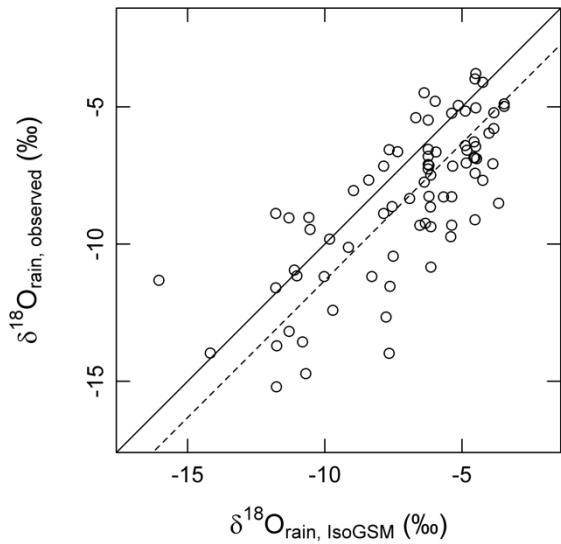


Figure S3: Relationship between the $\delta^{18}\text{O}$ of rainwater collected at the experimental site ($\delta^{18}\text{O}_{\text{rain, observed}}$) and the $\delta^{18}\text{O}$ of monthly IsoGSM predictions ($\delta^{18}\text{O}_{\text{rain, IsoGSM}}$). The solid line represents the 1:1 relation; the dashed line illustrates the mean difference between the two data sets ($-1.3\text{\textperthousand}$).

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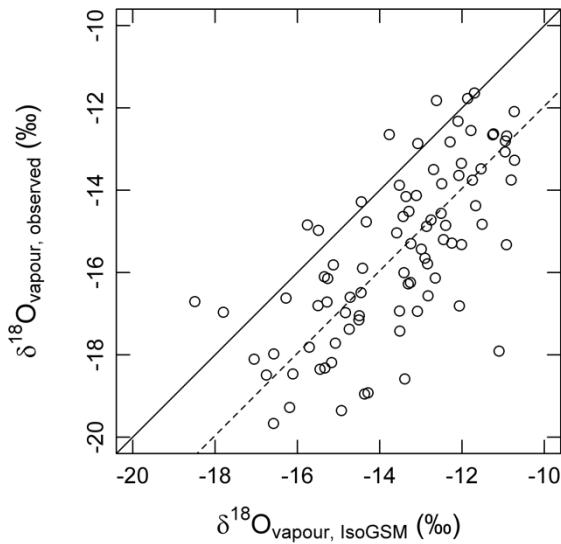
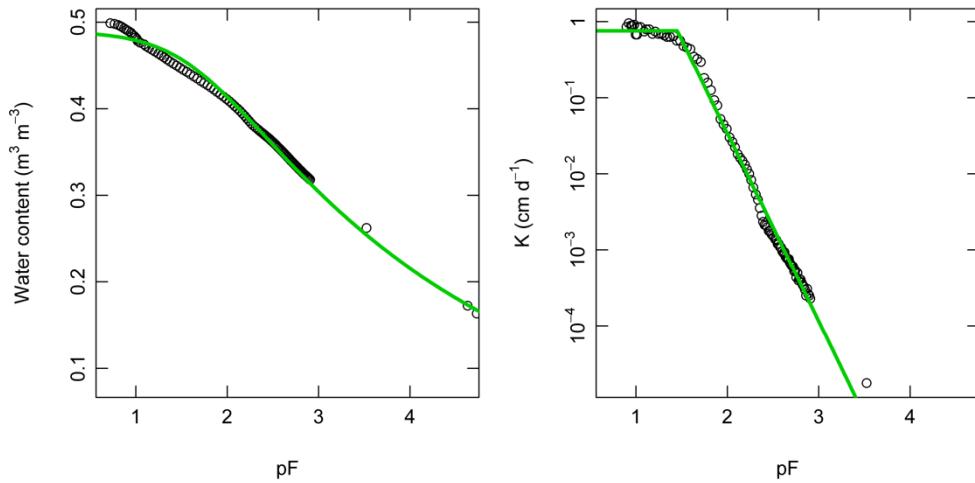


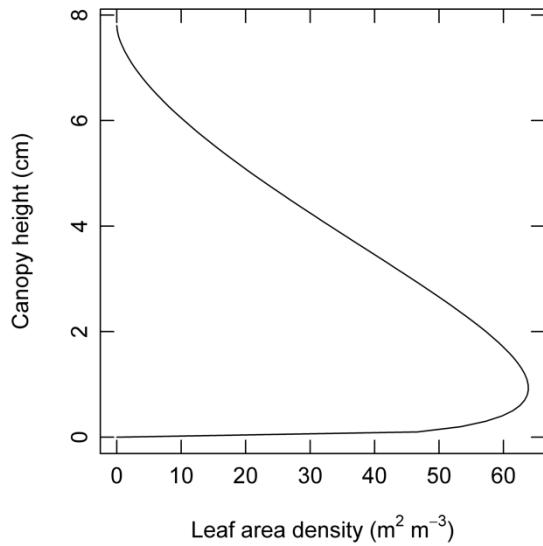
Figure S4: Relationship between the $\delta^{18}\text{O}$ of atmospheric water vapour as measured at the experimental site ($\delta^{18}\text{O}_{\text{vapour, observed}}$) and predicted by IsoGSM ($\delta^{18}\text{O}_{\text{vapour, IsoGSM}}$). The solid line represents the 1:1 relation; the dashed line gives the mean difference between the two data sets ($-2\text{\textperthousand}$).

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Figure S5: Relationship between volumetric water content (m^3 water m^{-3} soil) and pressure head, given as pF value (common logarithm of the pressure head in hPa), (left panel), and hydraulic conductivity (logarithmic scale) and pressure head (right panel), as derived from Hyprop measurements (open circles). The green curve in the left panel represents the Van Genuchten water retention curve fitted to the data, the green curve in the right panel shows the Brooks-Corey hydraulic conductivity curve fitted to the conductivity data. Derived parameter values are given in Table S1.



10 **Figure S6:** Beta distribution describing the assumed vertical leaf area density distribution at the experimental site (based on Wohlfahrt et al., 2003).

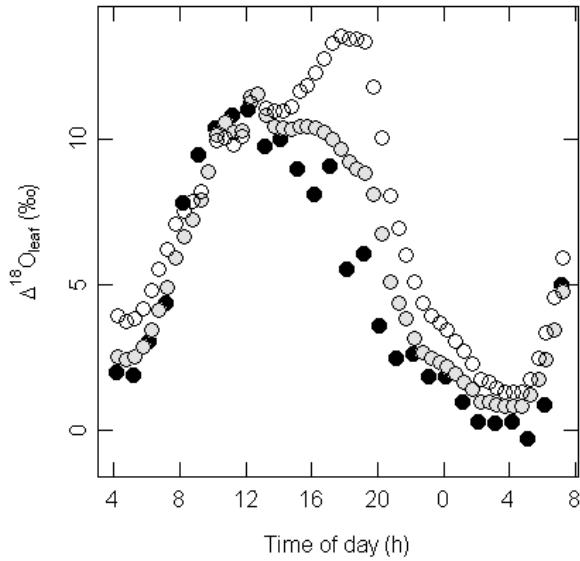


Figure S7: Diurnal time courses of ^{18}O -enrichment of leaf water ($\Delta^{18}\text{O}_{\text{leaf}}$) observed (closed circles) on 4/5 August 2005 in pasture paddock no.8 at Grünschwaige and predicted using the two-pool model with a constant proportion of unenriched water ($\varphi = 0.39$; grey circles) and the Pécelt model with a constant effective length ($L = 0.162$ m; open circles). Predicted and observed $\Delta^{18}\text{O}$ was calculated as the difference between $\delta^{18}\text{O}$ of leaf water and $\delta^{18}\text{O}$ of soil water at 7 cm depth. Observed $\delta^{18}\text{O}_{\text{soil}}$ at 7 cm depth was obtained from linear interpolation between the $\delta^{18}\text{O}_{\text{soil}}$ at 2 cm and 12 cm depth. Time is given in UTC.

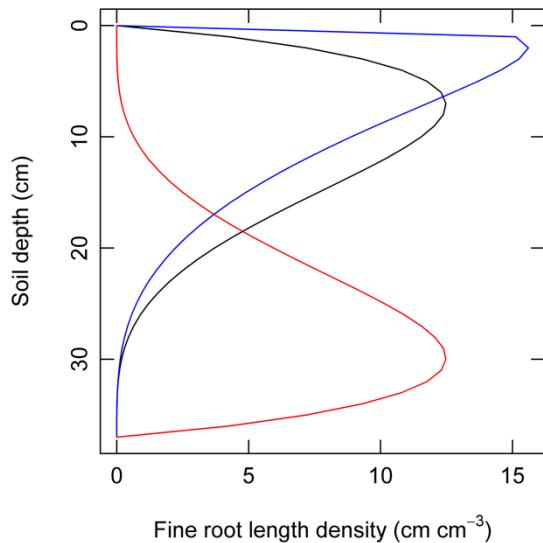


Figure S8: Beta distribution of fine root length density *versus* soil depth. The black line, with highest root density at 7 cm belowground, represents that used in the standard MuSICA runs; blue and red lines give the low and high alternative root distributions used in the sensitivity analysis (see Fig. 6h in main text), with maxima of root length density at 2 and 30 cm depth, respectively. All distributions have the same total fine root length (19 km m^{-2} soil surface).

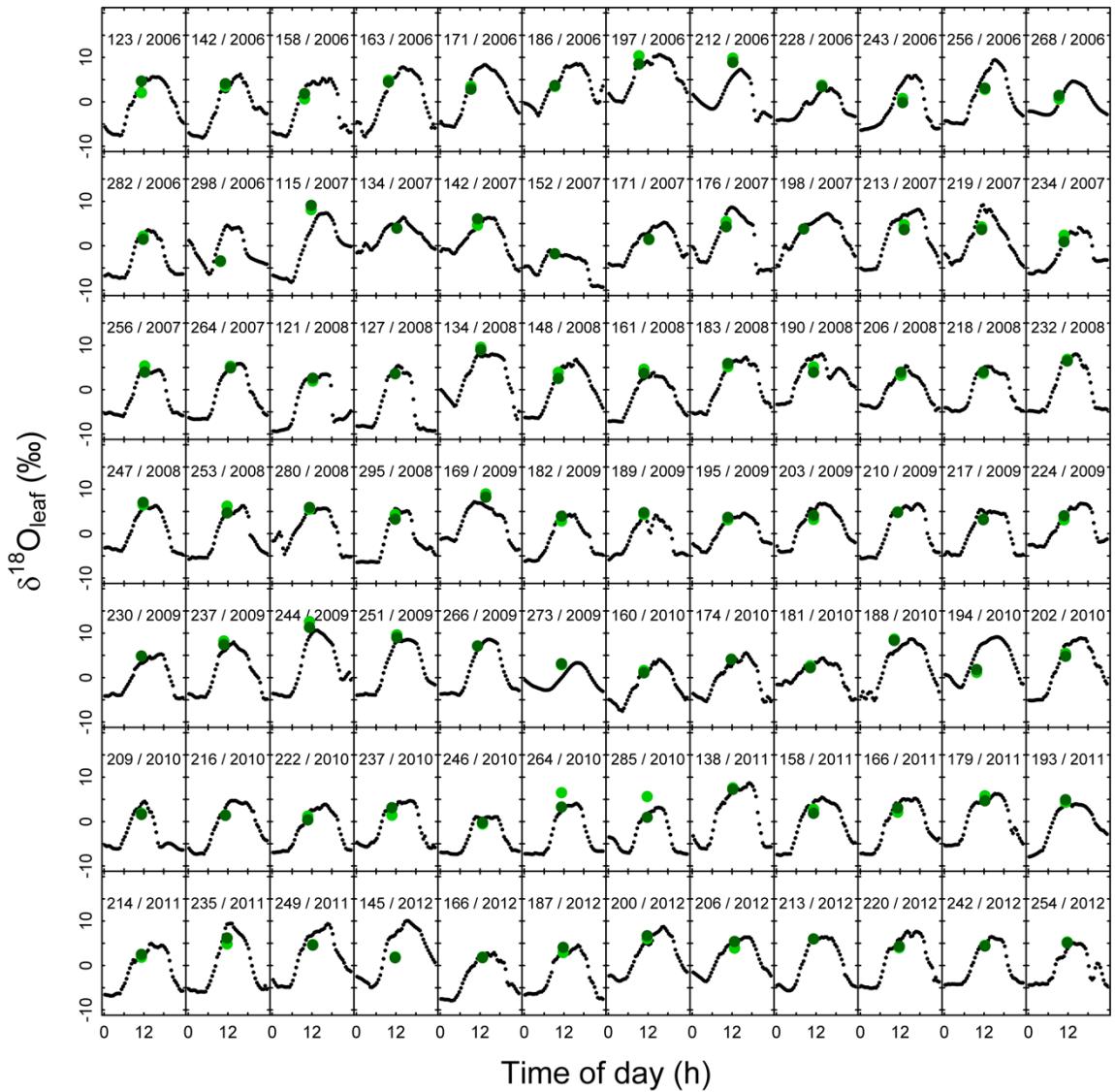


Figure S9: Diurnal cycles of modelled $\delta^{18}\text{O}$ of leaf water (black dots) and measured $\delta^{18}\text{O}$ of the two replicates of leaf water for all sampling dates (light and dark green dots). Numbers in the panels give the day of the year and year. Time is given in UTC.

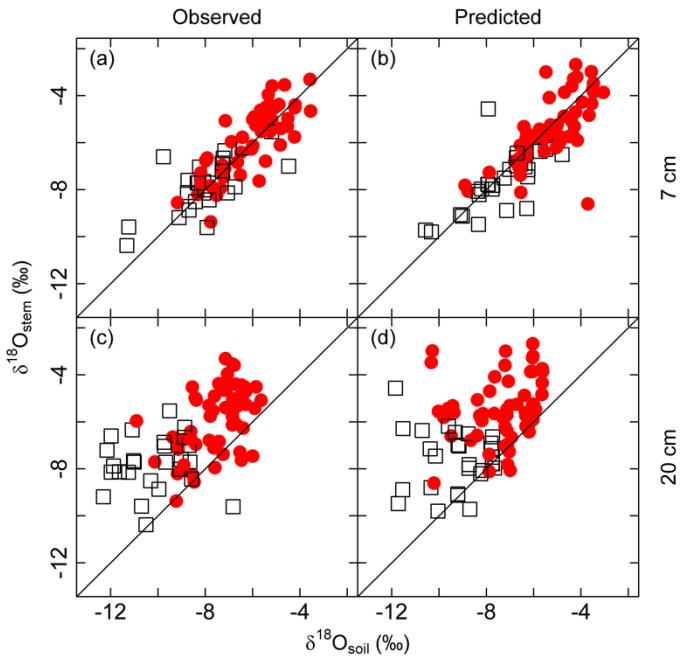


Figure S10 Correspondence between the $\delta^{18}\text{O}$ of stem water and soil water at 7 (upper panels) and 20 cm depth (lower) as observed (left) and predicted (right) in the first half (April to June; black squares) and in the second half of the vegetation period (July to October; red circles). The straight lines represent the 1:1 relationship.

5

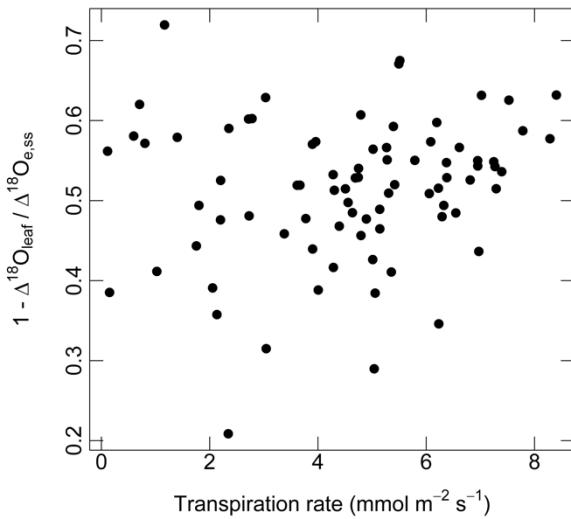


Figure S11: Relationship between canopy transpiration rate and the proportional difference between observed leaf water enrichment ($\Delta^{18}\text{O}_{\text{leaf}}$) and $\Delta^{18}\text{O}$ at the evaporative site, as predicted by the Craig-Gordon model ($\Delta^{18}\text{O}_{\text{e,ss}}$).

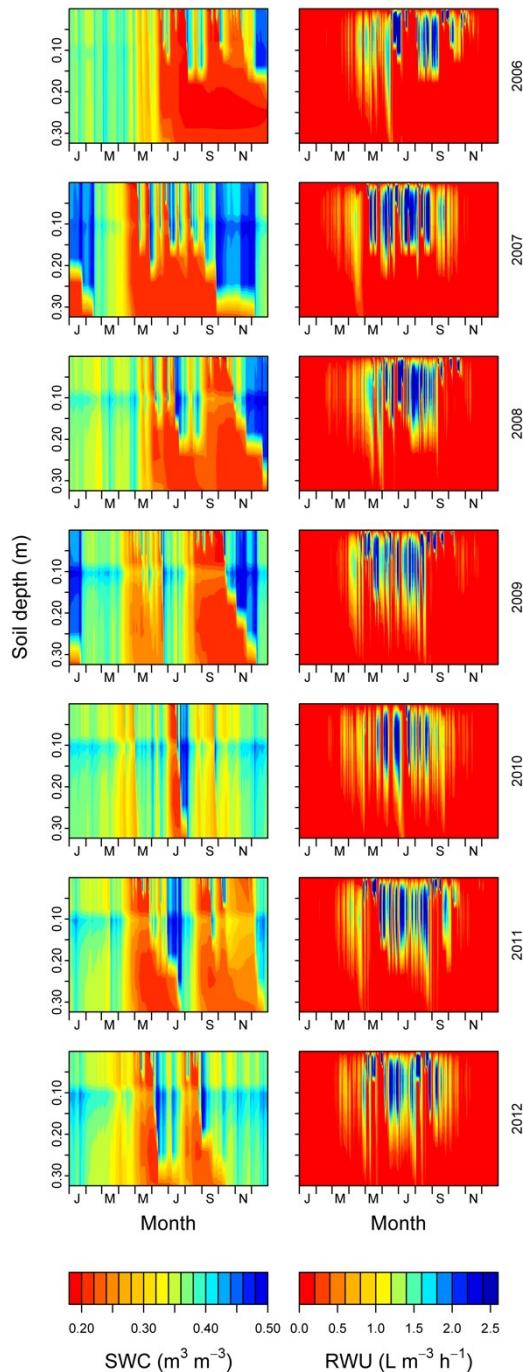


Figure S12: Soil water content (SWC) and root water uptake (RWU) along the soil profile as predicted by MuSICA for the studied period (2006-2012). The year is indicated on the right hand side.

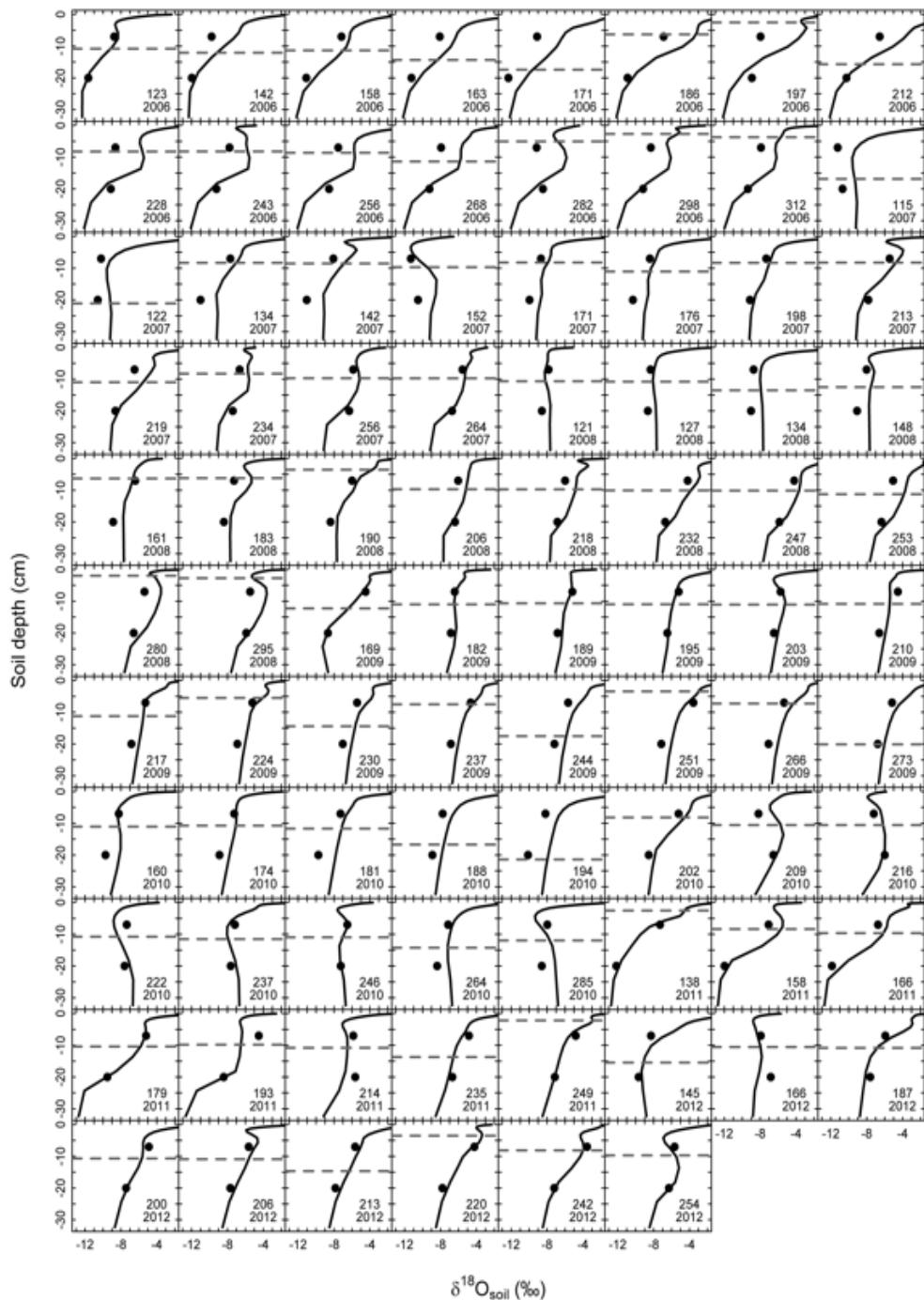
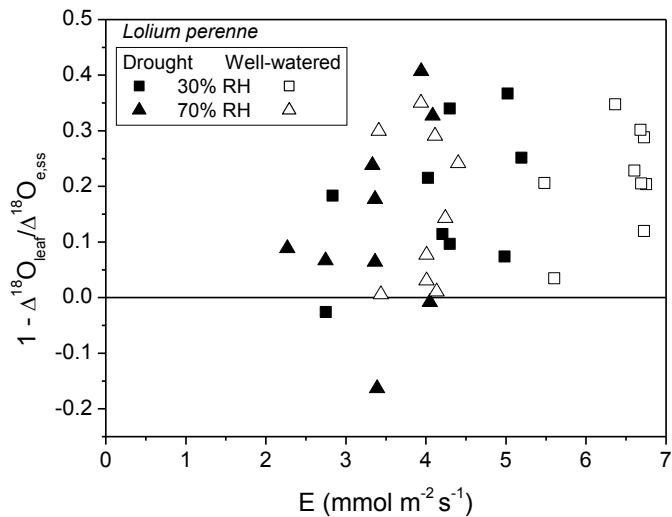
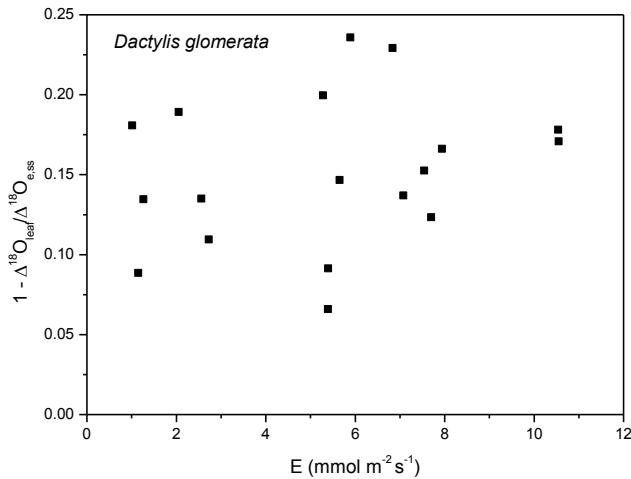


Figure S13: $\delta^{18}\text{O}_{\text{soil}}$ with soil depth as predicted by MuSICA (continuous lines) and mean uptake-weighted depth of root water uptake (dashed horizontal lines) on the different sampling dates. Closed circles: observations of $\delta^{18}\text{O}_{\text{soil}}$ at 7 and 20 cm depth. Sampling date is given by DOY and year, in the lower right corner of each panel.



| Figure S13S14: The relationship between transpiration rate (E) and the proportional difference between measured leaf water and the Craig-Gordon predicted enrichment ($1 - \Delta^{18}\text{O}_{\text{leaf}}/\Delta^{18}\text{O}_{\text{leaf}}$) for *Lolium perenne*. The relationship in Fig. S13 is statistically significant, but very weak: $1 - \Delta^{18}\text{O}_{\text{leaf}}/\Delta^{18}\text{O}_{\text{e}} = 0.017 E + 0.035$; $r^2 = 0.11$; $P = 0.045$.

5



| Figure S14S15: The relationship between transpiration rate (E) and the proportional difference between measured leaf water enrichment and that at the sites of evaporation ($1 - \Delta^{18}\text{O}_{\text{leaf}}/\Delta^{18}\text{O}_{\text{e}}$) within the leaf for *Dactylis glomerata*.