Author Comment (AC1): Response to Referee #1

We thank the referee for a careful and constructive review. During revision we made one substantial change. In addressing the reviewers' central concern about the fixed internal anatomy of the root cross-section (R1 SC1; R2 C2), we concluded that the rigorous solution was not to bound the original xylem/tissue model with a sensitivity analysis, but to revise the transport model itself. The revised model (Sect. 2.4) represents the cross-section as a well-mixed stele coupled to a surrounding cortex, with the stele–cortex exchange fixed from the independently measured night-time diffusion coefficient rather than fitted. Critically, the quantities we now report, radial uptake per unit root length (j_r) and axial flow rate ($Q_{x,0}$), are volumetric fluxes that do not depend on how the conducting area is distributed within the cross-section. We reference this revision throughout the responses below.

Referee comments are shown in italics, and our response is in blue

General comments

Comment 1: *The manuscript describes an interesting study that tries to analyze the effect of soil texture and soil water content (SWC) on the uptake and transport of D2O by crown and seminal roots of maize plants. The data analysis depends on a partly established model for uptake of D2O using neutron radiography. The observed variations/changes in D2O transport among root types on changes of soil water content are interpreted as changes in radial and axial fluxes. The authors conclude that fluxes are mainly influenced by soil texture especially for crown roots, more so than soil water content (SWC) but do so while assuming a fixed internal anatomical organization of the root cross-section, aside from differences in overall root diameter, even though the total flux (measured via weight loss) is nearly 30% less for loamy soil and more than 60% for sandy soil. Root lengths were also affected but less so than transpiration. A rough estimate shows that the changes in transpiration and root lengths do not completely match up with changes in axial and radial fluxes, about 20 to 25%. This is not discussed.*

Response 1: We thank the referee for this accurate summary of the study. We agree that the relative conductive fraction may itself vary with root type and soil environment, and that this was not tested in the original version. On examining this, we found that the original model was sensitive to the assumed conductive cross-section, and we therefore revised the model rather than merely bounding it. In the revised model the reported fluxes are volumetric quantities that do not depend on the internal conductive geometry, and the one remaining stele-fraction assumption only rescales the absolute values while leaving the between-treatment differences that underlie our conclusions unchanged. We address this in full under Specific Comment 1.

On the mismatch of roughly 20 to 25% between the changes in transpiration and root length and the changes in the modeled fluxes, we agree that this was not discussed, and we will add it now in the discussion. The mismatch is expected for two reasons. First, the reported fluxes are local values for segments 7 to 13 cm from the collar, and radial flux is not uniform along the root axis, as our own data show, so local fluxes need not equal the

axis-averaged uptake that would close against whole-plant transpiration. Second, the reported root length includes only crown and seminal root axes; lateral roots, which contribute to uptake, were not segmented and are therefore absent from this balance. We treat this more fully under General Comment 1.

On the strong difference in total transpiration among treatments, this reflects the integrated regulation of plant size, architecture, and hydraulics in response to texture and moisture. Our analysis concerns how the resulting uptake is distributed between root types and along the root, which is not determined by the total. We have made this distinction explicit in the revised discussion.

Comment 2: *Neither are the changes in shoot sizes discussed which may severely impact radial and axial fluxes which I think should be addressed more fully. This may partly be caused by ignoring potential changes in root anatomy caused by soil texture and/or low SWC within the model (see my point 1 in the specific comments).*

Response 2: We thank the referee and have added a paragraph on shoot development to the discussion. We now make the mechanistic link explicit: shoot size sets the transpirational demand of the plant, which sets the driving force for root water uptake, so treatment differences in shoot size are expected to propagate into the radial and axial fluxes.

On the referee's second point, that this may be confounded with unmodelled changes in root anatomy: the two effects act in parallel but are separable in principle, the shoot-size effect operating through transpirational demand on the whole root system and the anatomical effect through the conductive cross-section of individual root types. We now address the anatomical pathway directly through the model revision described under Specific Comment 1, in which the reported volumetric fluxes no longer depend on the assumed conductive cross-section; the demand-driven pathway therefore remains the more likely explanation for the observed changes.

Changes in the manuscript: a paragraph on reduced growth and transpiration and its link to transpirational demand was added as Sect. 4.1 (added in lines 482–490); the reconciliation of whole-plant transpiration with the local segment fluxes is given in Sect. 4.5: "transpiration integrates uptake over the entire root system, whereas our estimate captures only the analyzed axes and their attached laterals" (added in lines 591–599).

Comment 3: *In addition, a more extensive discussion on the changes of local SWC due to the D_2O injection is required as this disturbs the root water uptake pattern, the measurement is a perturbation, especially in sandy soil that is drier and therefore experiences a larger local SWC change.*

Response 3: The referee is correct on both the effect and its texture dependence, and we have expanded the discussion accordingly. We would add one design feature that limits the perturbation. During the experiments the capillary barriers on both sides of the sample were left open, so that water potential could equilibrate across the whole soil rather than being confined to the injected compartment. This reduces the magnitude and duration of the local increase in soil water content that follows the injection.

Changes in the manuscript (Sect. 4.5): the perturbation is now discussed in full — "Because a matric-potential perturbation propagates through connected soil by hydraulic (pressure) diffusion, it re-equilibrates faster than the water content itself…", and "the ~75 mL added per compartment is only ~4.5 % of the entire container… the ~2 h measurement window was far shorter than the ~24 h over which local uptake is reported to respond to soil-water change (Ceolin et al., 2025)" (added in lines 600–614).

Specific comments

Specific Comment 1: *I am perfectly fine with the idea that the model is a significantly simplified version of reality. However, this simplification very likely comes with a major issue. Here, internal root anatomy is assumed fixed, e.g. a constant 5% conductive xylem fraction, a constant not derived from microscopy data. But seminal roots and crown or nodal roots typically do not have the same relative conductive area and this area is not constant over time (the changing fraction of early metaxylem and late metaxylem over time). More importantly, xylem organisation with the roots changes on soil texture and especially soil water content. See for a recent article with references: https://doi.org/10.1002/csc2.70084 by Yutong Jiang and JK Whalen. Root anatomy can change substantially (e.g. changes in stele diameter, changes in xylem bundle numbers, xylem sizes) with conditions but when they do the model used by the authors does not allow for this as it is presented here. This implies that anatomical changes upon variations in soil texture or SWC are interpreted in terms of changes in axial and/or radial fluxes. At the moment it is completely unclear how this affects the modelling results of this manuscript.*
So, when I pose a counter-hypothesis stating that anatomical variations/changes between CR and SR upon differences in soil texture and SWC cause the observed changes in D2O fluxes, how would the authors provide proof that this alternative hypothesis is less likely to be correct.
What is needed therefore is at minimum an analysis where the effect of changes of Ax, the xylem stele diameter, especially in relation to overall root diameter are modelled, basically a sensitivity analysis, such that the importance of anatomical variations can be at least somewhat quantified. Better, would be to perform microscopy on roots that have been grown under the investigated experimental conditions and quantify these anatomical changes and insert these into the model.

Answer to Specific Comment 1: We thank the reviewer for this important comment, which prompted the most substantial improvement to the manuscript. The reviewer is right: on examining this concern we found that the original model was indeed sensitive to the assumed conductive cross-section, so that a fixed conductive fraction could bias the inferred fluxes. Following the reviewer's suggestion, we therefore revised the model itself rather than merely bounding it with a sensitivity analysis. The revised model (Section 2.4) represents the root cross-section as two coupled domains: a well-mixed stele, the inner conducting domain through which the labelled water is transported axially, and a surrounding cortex, which fills by radial diffusion and is diluted by the radial uptake of soil

water. The two domains are coupled by an exchange coefficient that is not fitted but computed from the root radial diffusion coefficient measured independently in the night-time experiments. This structure is anatomically motivated: in maize, the xylem conduits are distributed as an array across the stele over sub-millimeter distances, so diffusion equilibrates the stele within seconds and the well-mixed assumption is justified, while the dominant radial resistance is located at the endodermis, which the exchange coefficient represents.

Crucially, the quantities we now report (the radial uptake per unit root length (jr) and the axial flow rate (Qx,0)) are volumetric fluxes set by the tracer mass balance, and are therefore independent of how the conducting area is distributed within the cross-section. The counter-hypothesis raised by the reviewer, that anatomical differences in vessel number, size, or stele diameter drive the apparent flux differences, does not apply to a volumetric flow rate. One geometric assumption does remain — the fraction of the cross-section assigned to the well-mixed stele, which we fixed at 40 %. Although this remains an assumption, it is no longer crucial: it only rescales the absolute fluxes, and because the same value was applied to every root it leaves the between-treatment and between-root-type contrasts, on which all our conclusions rest, essentially unchanged. We state this explicitly in Section 4.5 and acknowledge there that treatment-dependent anatomical variation is not resolved by the model. We agree that microscopy on the same plants would be the most direct test and would allow the measured anatomy to be embedded in the model; this material was not preserved for sectioning after the beamtime and is therefore unavailable, which we now state as a limitation, identifying the combination of neutron radiography with quantitative root anatomy in a functional-structural framework as the necessary next step.

Changes in the manuscript: the revised two-compartment (stele–cortex) model is set out in Sect. 2.4 (added in lines 206–252) and parameterized in Sect. 2.5. We have now also added a new section 4.5: “Methodological considerations and model assumptions) to detail our model fluxes and their limitations and interpretations (added in lines 590–644). Note that the new model resulted in slightly different values of radial and axial fluxes as well as diffusion coefficients, as the previous one was cross-section dependent.

Specific Comment 2: The authors should explain why they chose this specific form of Eq.7 which is unclear to me. Also explain what to do when *a* turns negative. How is this handled or is this an impossibility and $az > -1$ always imposed? (otherwise, the solution is imaginary if *b* is a non-integer?) Also, doesn't *a* have the dimension of 1/cm or 1/mm or 1/m, the inverse of *z* (not mentioned)? In case *b* is odd, the flux can be negative is $az<-1$ which I also find difficult to interpret. Maybe the authors can expand a bit on this.

Answer to Specific Comment 2: We thank the reviewer for these detailed questions, which overlap with a comment from Referee 2, and we have rewritten the description of Eq. (7) in Section 2.6. On the choice of form, Eq. (7) is an empirical two-parameter family, not a mechanistic law. It was chosen because two parameters are sufficient to represent the axial flux profiles that are physically plausible over a segment of this length: with b = 0

the flux is constant and radial uptake is zero. On the sign of the shape parameters: in the revised model a and b are constrained to be non-negative (a, b ≥ 0), which guarantees that the axial flow is non-decreasing along the segment and hence that the radial flux is non-negative — water is taken up, not released, above the capillary barrier. With a ≥ 0 the argument (1 + az) is always positive over the segment, so the solution is real for any b and negative or imaginary fluxes cannot arise. This constraint was imposed through the bounds of the optimization.

Changes in the manuscript (Sect. 2.6; the reviewer's Eq. 7 is Eq. 8 after renumbering): "a and b were constrained to be non-negative (a, b ≥ 0), so that the axial flow was non-decreasing and the radial flux jr therefore remained non-negative" (added in lines 298–301). And also detailed in the parameterization section as "he search ranges were $Q_{x,0} \in [10^{-6}, 10^{-1}]$ cm^3 min^{-1}, a ∈ [0, 2] and b ∈ [0, 6]; the non-negativity of a and b guarantees a physically admissible (non-negative) radial-flux profile." (in lines 313-315).

Specific Comment 3: *The authors mention that the lower boundary condition for tracer concentration entering the root segments is assumed, not measured. This may influence the fitted concentrations and so the fluxes. This is a point that requires some discussion.*

Answer to Specific Comment 3: We agree this assumption should be justified more fully, and we have expanded the text in Section 2.5. The lower boundary concentration was set to the saturated tracer value, and two features of the setup ensure this represents the actual inlet condition rather than an arbitrary imposition. First, the fitted region does not start at the H_2O–D_2O mixing front itself but along the root approximately 2 cm above it, from that point to z = L, while the simulation is performed right at the H_2O–D_2O mixing front. The lower boundary of the fitted region, therefore, lies where the xylem tracer concentration has already reached its plateau. Second, fitting begins only once the tracer front has propagated past this lower boundary, approximately 4–8 minutes after injection, so that the inlet concentration has stabilized before the simulation starts. Together, these ensure that the assumed boundary value corresponds to the established, rather than the transient, inlet concentration.

Changes in manuscript: The description and justification of the lower boundary condition have been expanded in Section 2.5 (lines 279-293).

Specific Comment 4: *Another issue is that it is not clear whether the authors looked at seminal and crown roots that include the lateral roots or not. Judging from the reported average root diameters this might not be the case. This point needs to be clarified. Also, did the authors verify that the measured root lengths per NT are the same or similar to standard measurements looking at excavated roots whose lengths have been quantified? For a older than 45 days maize plant (or is it 6 weeks?) root lengths of less than 10m appear quite low to me, I would have expected a root length of more than 30m or so. Lateral root diameters are significantly less than primary axis roots, so how is this treated in the model?*

Answer to Specific Comment 4: We analyzed crown and seminal root axes only; lateral roots were not included. As the reviewer inferred from the average diameters, the fine

laterals are excluded. They could not be segmented reliably from the neutron radiographs because their diameter is at or below the spatial resolution and their contrast against the wet soil is insufficient. We have stated this explicitly in the manuscript (Sect. 4.1 and 4.5).

Changes in the manuscript: that the analysis excludes fine lateral roots is stated in Sect. 4.1 — "our study excluded lateral roots, which can contribute substantially to total root length" (line 488-490); the whole-axis interpretation of jr (which includes water entering through the attached laterals) is given in Sect. 4.5: "they represent the total radial influx per unit length of the main axis, including the water entering through the laterals attached to that axis …" (added in lines 598–599).

We note, however, that although lateral roots are absent from the measured root length, the radial flux we report is defined per unit length of the main axis and effectively includes the water entering through the laterals attached to the analyzed segments; the laterals therefore contribute to the estimated uptake even though they are not resolved as separate objects.

This also explains the apparent discrepancy in total root length. The lengths we report are cumulative lengths of crown and seminal axes, not whole-root-system lengths. Lateral roots typically account for the majority of total root length in maize at this stage, so a whole-system length in the range the reviewer expects (> 30 m) is possible.

We did not cross-validate the neutron-derived axial lengths against excavated root systems for these plants. The measurements are internally consistent across treatments, and because laterals are excluded by design, they are not directly comparable to standard whole-system excavation lengths.

The plant age is 45 to 47 days; we have corrected the inconsistent "six weeks" wording throughout (see also Specific Comment 6).

Specific Comment 5: *It is not clear from the experimental section how many plants were used per condition. In the Fig.3 caption it is indicated N=6 so I suppose 24 plants were measured in total, correct? This needs to be mentioned clearly in the methods section. How many segments of CR and SR were measured per plant and how where these segments treated in the statistical analysis? What about the variation among individual segments among individual plants for both CR and SR as compared to variation between plants? Also, what are the estimated error margins for the modelled parameters, based on the assumption that the model assumption themselves are correct? It is not clear to me how the in-between plant variations compare with the in-between segments and within-model variations. So, how are the segments treated, as independent observations or not as this may lead to pseudoreplication?*

Answer to Specific Comment 5: We thank the reviewer for raising these important points on replication and pseudoreplication, which we now address explicitly in the revised manuscript.

(i) Number of plants. The reviewer is correct that plant-level growth traits (Fig. 3) were measured on five plants per treatment (20 plants in total). We have clarified, however, that

the D_2O-based root-water-uptake modelling was performed on a subset of 12 of these plants that were imaged by neutron radiography, as beam time did not permit imaging all 20 plants. This subset comprised three plants per treatment. This is now stated in the Methods (Section 2.2).

(ii) For each imaged plant, all crown and seminal root segments crossing the analyzed compartments were modeled individually (on average, nine crown segments per plant, range 4–12, and four seminal segments, range 1–7). Because multiple segments per plant are not independent, we did not treat them as independent replicates. Instead, radial flux and axial flow rate were analyzed with a linear mixed-effects model with texture, moisture, and their interaction as fixed effects and plant identity as a random intercept; treatment effects are thus tested against between-plant variation, and within-plant pseudoreplication is accounted for. This is now described in the Methods and stated in the Fig. 5 caption.

(iii) We now report the variance partition from these models (Section 3.5 in lines 446-454) as follows: "The mixed-effects models also partition the treatment-independent variability into a between-plant and a within-plant (between-segment) component. In crown roots, plant identity accounted for a substantial share of this variability (≈27 % for j_r and ≈29 % for $Q_{x,0}$), indicating consistent differences among individual plants; the significant soil-texture effect therefore held even after this between-plant variation was removed. In seminal roots, the between-plant component was small and could not be reliably resolved given the few plants available, so most of the unexplained variability in seminal roots occurred among individual segments rather than among plants; accordingly, the absence of a detectable treatment effect in seminal roots reflects, in part, limited replication and should be interpreted with caution. This is also further discussed in the discussion section in lines 538-544.

Specific Comment 6: *It is reported that the plants were older than 45 days (!! in the methods section, the abstract says six-weeks-old which is somewhat inconsistent), but as you measured 24 plants, I assume, and each measurement took at least 3h or so, what was the difference in age of the plants? I ask this as the metaxylem of maize plants develops over time (late metaxylem becomes more important over time).*

Answer to Specific Comment 6: The reviewer is correct on both the inconsistency and the underlying concern, and we have addressed both. In terms of age, we have made the wording consistent throughout. Plants were 45 to 47 days old at the time of measurement: cultivation to the start of imaging took 45 days, and the labeling experiments were carried out over a two-day period.

On the number of plants, the D_2O labeling during daytime was performed on three plants per treatment, that is, 12 plants in total. Because the campaign spanned two days, the maximum difference in age among the measured plants was two days. This is now stated in the Methods section (lines 169-170).

Technical comments:

Equation 8 is a rearrangement of Equation 3 and is thereby somewhat redundant.

Answer to Specific Comment 6: We agree with this rearrangement of Eq. 3 and have removed it now.