

Reviewers 2

General comments:

The manuscript presents a work on the effect of soil texture and soil moisture content on hydraulic of crown roots (CR) and seminal roots (SR) of Maize. Neutron radiographs tracked deuterated water (D2O) concentration changes in roots after injection of D2O into soil at a given point close to the root. Root hydraulic was studied in two soil types, loamy (L) and sandy (S), and under two moisture conditions, well-watered (W) and water-limited (D) treatments. The data analysis is based on model inversion. The model consists of two domain, a xylem domain (x) and a domain surrounding the stele (t). In each domain, D2O concentration is governed by a diffusion-convection equation with terms accounting for transfer between domains and/or soil and root depending on the domain considered. Main results are:

- soil texture had a stronger impact on hydraulic of crown roots than soil moisture;*
- crown and seminal roots had “contrasting hydraulic behavior”;*
- hydraulic architecture had an important impact on hydraulic.*

The experimental work is very good as it was in the past works. The topic is in the scope of Hydrology and Earth System Sciences.

Response: We thank the reviewer for the positive assessment of the experimental work and for judging the study to be within the scope of Hydrology and Earth System Sciences. We are grateful for the detailed and constructive comments that follow, several of which improved the manuscript, and we respond to each of them below.

However, as general comments and in my point of view:

Comment 1: *the manuscript does not present clear added value or novelty to previous works or to existing literature.*

Response 1: *This work technically and methodologically builds on our previous works, but it addresses a substantially different and novel question that has not been quantified in the literature so far: how local root water uptake along different root types and root lengths responds to soil texture and moisture. Therefore, the objectives and findings of this MS are novel and presented for the first time.*

Changes in the manuscript: the novel question is stated in the objectives (Sect. 1): “quantify differences in RWU patterns between soil types and assess the influence of water availability on uptake dynamics... and... quantify how RWU varies along the root axis as a function of distance from the tip” (in lines 74–83).

Comment 2: I think that anatomical data are missing to be able to analyze observed differences between root types and soil types. Or the use of a function-structure plant model.

Response 2: We agree, and this is the same concern raised by Reviewer 1 (Specific Comment 1), where we address it in full. We summarize our response here.

Anatomical data for these plants are not available. The material was not preserved for sectioning after the beamtime, so we cannot insert measured anatomy into the model. We would note, however, that a constant conductive fraction does not mean a constant xylem radius. We measured root diameter in each treatment and used these values in the model, so the absolute conductive area already varied between treatments in proportion to the measured diameter.

On examining this concern we found that the original model was sensitive to the assumed conductive cross-section, and we therefore revised the model itself (new Section 2.4) rather than bounding it with a sensitivity analysis. The revised model represents the cross-section as a well-mixed stele coupled to a cortex, and the quantities we now report (the radial uptake per unit root length (j_r) and the axial flow rate ($Q_{x,0}$)) are volumetric fluxes that do not depend on how the conducting area is distributed within the cross-section. The alternative hypothesis, that anatomical differences drive the apparent flux differences, therefore does not apply to these volumetric quantities. The one remaining geometric assumption, the stele fraction, only rescales the absolute values and, because it is applied identically to every root, leaves the between-treatment and between-root-type contrasts unchanged. This is given in full in our reply to Reviewer 1, Specific Comment 1.

Changes in the manuscript: the revised stele–cortex model is described in Sect. 2.4 (added in lines 214–252) and its assumptions are discussed in Sect. 4.5 (lines 591–644); see the pointer under Reviewer 1, Specific Comment 1.

On the point of using a function-structure plant model, we previously developed such a model in our previous works, and it needs several parameters at the single root levels (endodermis and cortical cells). In this work, we discussed using such a simplified model to ensure that our inverse modeling will not be ill-posed due to over-parameterization. We will clarify this now in the main text.

Comment 3: The explanation of equation 7 did not convince me, see specific comments.

Response 3: We agree that the original justification was too thin. Reviewer 1 raised the same equation, and our replies are consistent.

Equation 7 is an empirical two-parameter family, not a mechanistic law. It was chosen because two parameters are enough to describe 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. We add the two points raised on the same equation. The parameter a has the dimension of inverse length (cm^{-1}), so that the product az is dimensionless. In the revised

model a and b are constrained to be non-negative, 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 $a \geq 0$ the argument $(1 + az)$ remains positive over the segment, so the solution is real for any b and negative fluxes cannot arise. (detailed in section 2.6 lines 298-301 and 313-215)

Specific comments:

Equations 1 and 2:

- may be a quick description about how these equations were derived would be of interest. In particular to explain the division by A_x and A_t .

We have added a short test to support derivation of equations in Section 2.4 as follows “Both Eq 1. and Eq. 2 are written per unit volume of their compartment. Because the concentrations C_s and C_c are amounts of tracer per unit volume, each cross-section-integrated transport term is divided by the corresponding cross-sectional area to yield a rate of change of concentration: the axial advective–diffusive divergence in the stele is divided by A_s (Eq. 1), and in the cortex (Eq. 2) the stele–cortex exchange carries the factor A_s/A_c , ensuring that the tracer leaving the stele ($\propto A_s$) equals the tracer entering the cortex ($\propto A_c$), while the radial-uptake term is divided by A_c because the water entering across the root surface ($2\pi r j_r$ per unit length) dilutes the whole cortex volume. (added in liens 229-235)

- in the results, it is never question of the exchange rate k_{xt} , or I missed it, and it is one of the optimized parameter. One sentence about it would be of interest.

The stele–cortex exchange coefficient is no longer a fitting parameter in the revised model: it is computed from the root radial diffusion coefficient (D_r) measured independently in the night-time experiments, when axial flow is negligible (Section 2.4, Eq. 3). The division by the stele and cortex cross-sectional areas in Equations 1 and 2 arises because each mass balance is written per unit cross-sectional area of its compartment; we have added a brief derivation. The only fitted parameters are those describing the axial-flow profile ($Q_{x,0}$, a, b).

Changes in the manuscript (Sect. 2.4, Eq. 3): the exchange coefficient (denoted k_{sc} ; the reviewer’s k_{xt}) is now derived, not fitted — “The two radial exchange rates were not fitted. They were derived from the root diffusion coefficient measured independently in the night-time experiments...” (added in lines 269–273); an area-normalized derivation of Eqs 1–2 was added — “each cross-section-integrated transport term is divided by the corresponding cross-sectional area to yield a rate of change of concentration...” (added in lines 229-235).

Specific comment 1: Equation 7: Why this particular profile chosen? While I understand that it simplifies the calculation, analytical solution exist, see for instance Landsberg and Fowkes (1978). Because this profile has probably a strong impact on the analysis, I would

recommend that the authors provide a stronger justification for this choice. Simplification is, I think, not sufficient.

Response: This comment is addressed in our responses to comment 3.

Specific comment 2: Table 2: how many plants were used per values? Is that 6 individuals as in figure 3?

Response: The values in Table 2 are means and standard deviations of five plants per treatment. Of these five, three plants per treatment were later used for the D2O labelling experiments. We added this to the Table 2 caption and made the replication consistent across the Methods, Table 2, and Fig. 3.

Specific comment 3: Fig. 4: Which diffusion coefficient is it? D_x or D_t ?

This is the effective radial diffusion coefficient of the root tissue, now denoted D_r , obtained from the night-time experiments. The notation has been made consistent (D_r) throughout.

Changes in the manuscript (Sect. 3.4, Fig. 4): the coefficient is the “effective radial diffusion coefficient of the root tissue (D_r)”, obtained from the night-time experiments and denoted D_r throughout (added in line 269-273).

Discussion:

Specific comment 4: As I wrote in the general comment I do not see, according to the different paragraphs, what this work provides as new or complementary results according to existing literature.

Response: We addressed this in our reply to General Comment 1.

L, 425: “Classical soil–plant hydraulic theory predicts that root water uptake scales with both soil hydraulic conductivity and the water potential gradient between the soil and the root (Passioura, 1988; Steudle, 1994).” This assertion is no true, at least not anymore. The references are too old. Just have a look for instance to: Doussan2006 where he clearly showed the importance of the root hydraulic architecture; Cai2022 regarding the root-soil contact, where by the way the soil structure can be important; or Javaux2008 about the evaporative demand. And there are many other references. In the following the authors are exactly writing that. Therefore, the authors should not, present there results as a novelty, as I had the feeling in this paragraph.

Response: We thank the reviewer and have addressed both parts of the comment — the outdated statement with its references, and the impression that an established concept is presented as novel.

On the statement and references. We agree that root water uptake is not adequately described by the classical relation (uptake $\propto$ soil hydraulic conductivity $\times$ soil–root potential gradient), and that Passioura (1988) and Steudle (1994) are not the appropriate references for the current understanding. We have removed that sentence. The paragraph no longer asserts this relation as theory; instead we introduce it explicitly as a first-order,

bulk-soil expectation — a baseline that our data then contradict — and we attribute the modern understanding to the references the reviewer suggests. The revised text reads: "A first-order expectation based on bulk soil properties alone would be that radial uptake scales with soil hydraulic conductivity and with the water potential gradient between soil and root, and would therefore be highest in wet loamy soil... Our results showed the opposite... It has long been established that bulk soil hydraulic properties alone are insufficient, and that root hydraulic architecture (Doussan et al., 2006; Zarebanadkouki et al., 2014b), root–soil contact and soil structure (Duddek et al., 2022), and evaporative demand (Javaux et al., 2008) co-determine uptake." (revised, lines 574–583). Following the reviewer's pointer, we have also added Cai et al. (2022) to the root–soil-contact citation. (Detailed in lines 506-5023)

On novelty. The contribution of this study is empirical, not conceptual: a direct, in-situ, root-type-resolved quantification showing that local radial uptake in crown roots is controlled by soil texture in the direction opposite to the bulk-soil expectation (dry sand exceeded wet loam), while seminal roots remain insensitive. To our knowledge, this has not been measured before. We have adjusted the wording so the paragraph frames the observation, not the concept, as the new result. (detailed in lines 75-84).

§ 4.2: "These findings align with previous studies" is the beginning of the paragraph showing again the lack of novelty.

We thank the reviewer for this comment and take the opportunity to clarify where the novelty of this study lies. We agree that our methodology builds directly on our previous work — the D₂O tracer approach and the diffusion–convection modelling framework are established, and we make no claim of methodological novelty.

The novelty of the present study is instead in the research question and the findings: to our knowledge, this is the first time that root water uptake has been resolved at the level of root type (crown vs. seminal) as a function of both soil texture and soil moisture within the same experimental system. The central results, that crown-root radial uptake is governed primarily by soil texture rather than moisture, and in a direction opposite to a bulk-soil-conductivity expectation (higher in sand than in loam), while seminal roots remain comparatively insensitive, have not been reported before and are the contribution of this work.

Changes in the manuscript (Sect. 4.3): the sentence now reads "The greater axial capacity of crown roots is consistent with well-documented anatomical and hydraulic differences between maize root types," citing earlier work in support of our result rather than as its premise (added in lines 571–580).

§ 4.4: L. 498-499 the assertion "axial flow is directly proportional to the conductive area (i.e., $\propto \pi r^2$)" is known to be erroneous see for example the review Barry2025 and references in it. The authors should revise this statement.

We thank the referee for pointing us to Barry et al. (2025). In the revised model this concern no longer arises: the axial flow rate ($Q_{x,0}$) is now a directly fitted volumetric

quantity, obtained from the propagation of the tracer front, and is not computed as the product of an axial flux and an assumed xylem area ($Q = j_x \cdot \pi r_x^2$). The criticized proportionality, therefore, no longer appears in the manuscript. However, we still used this reference and acknowledged (in Section 4.3) that axial hydraulic conductance depends on vessel number and the distribution of vessel radii rather than on total conductive area (Barry et al., 2025).

Changes in the manuscript: the statement that axial flow is proportional to conductive area no longer appears (that part of the Discussion was restructured, and the axial flow rate is now the directly fitted $Q_{x,0}$); the vessel-velocity point is made in Sect. 4.5 — “the true vessel velocity exceeds the velocity obtained by normalizing the flow to the whole stele area; this does not bias our estimates, because the reported quantities ... are volumetric fluxes” (added in lines 639–644).

L. 500-502 I agree with this point, that highlights, in my point of view, missing anatomical data or a limitation of the proposed modeling approach that do not account for the structure–function relationships that govern root hydraulics (see, for example, Boursiac et al., 2022).

We agree. Section 4.5 now discusses this limitation directly: the two-compartment representation prescribes a fixed geometry and does not fully resolve the structure–function relationships that govern root hydraulics, in which vessel number, the distribution of vessel diameters, and the development of apoplastic barriers jointly determine axial and radial conductance (Barry et al., 2025; Boursiac et al., 2022). The revised model reports volumetric fluxes that are robust to the assumed conductive fraction, but this cannot substitute for measured anatomy. Separating the anatomical from the physiological contributions to the observed differences would require combining neutron radiography with quantitative root anatomy on the same plants, translating the measured anatomy into hydraulic properties, and embedding the resulting root-type-specific hydraulics in a functional-structural plant model, which we regard as the necessary next step.

Changes in the manuscript (Sect. 4.5): the model’s fixed, two-compartment geometry is discussed as a limitation — “The transport model itself uses a simplified two-compartment representation of the root tissue that is nonetheless grounded in root anatomy...” (added in lines 615–627).

Technical comments:

l. 42: “branch perpendicularly” I think, it is not correct the angle is not always 90°. May be revised “perpendicularly”

Response: Corrected. The text no longer states that lateral roots branch perpendicularly; it now says they branch at variable angles.

l. 118: in Table 1 legend “)” is missing after θ_{vol} in “Volumetric water content (θ_{vol} ,”

Response: Corrected. The missing closing parenthesis was added.

l. 156: "during periods during the day" I guess that would be "during the day"?

Response: Corrected.

Fig. 2: According to the figure $A_t = \pi r^2$ not $\pi r^2 - A_x$ as explained l. 242.

Response: Corrected. The definition in the text is the correct one: the cortex domain excludes the stele, so $A_c = \pi r^2 - A_s$. The figure was inconsistent with this and has been corrected.

Fig. 2: Even if the legend is clear, panels (a) and (b) are not labeled on the figure.

Response: Corrected. Panel labels (a) and (b) were added to the figure.

l. 385-387: *Italic format but it should be roman. Unless this is part of the figure legend, therefore it should be in it.*

Response: Corrected. This was part of the legend, and we mistakenly broke into a new line.

Fig. 5: in legend it is written "right column (b, d, f) for SR" but there is no (f).

Response: Corrected.

References:

- Barry, Luke, Juan Carlos Baca Cabrera, Mikael Lucas, Guillaume Lobet, Yann Boursiac, and Alexandre Grondin. "Role of Xylem in Root Hydraulics: Functionality and Implications for Drought Adaptation." *Quantitative Plant Biology* 6 (2025): e42. <https://doi.org/10.1017/qpb.2025.10026>.
- Boursiac, Yann, Christophe Pradal, Fabrice Bauget, et al. "Phenotyping and Modeling of Root Hydraulic Architecture Reveal Critical Determinants of Axial Water Transport." *Plant Physiology* 190, no. 2 (2022): 1289-1306-1289-306. <https://doi.org/10.1093/plphys/kiac281>.
- Gaochao Cai, Mutez Ali Ahmed, The role of root hairs in water uptake: recent advances and future perspectives, *Journal of Experimental Botany*, Volume 73, Issue 11, 2 June 2022, Pages 3330–3338, <https://doi.org/10.1093/jxb/erac114>
- Doussan, Claude, Alain Pierret, Emmanuelle Garrigues, and Loïc Pagès. "Water Uptake by Plant Roots: II – Modelling of Water Transfer in the Soil Root-System with Explicit Account of Flow within the Root System – Comparison with Experiments." *Plant and Soil* 283, nos. 1–2 (2006): 99-117-99-117. <https://doi.org/10.1007/s11104-004-7904-z>.
- Javaux, M., Schröder, T., Vanderborght, J. and Vereecken, H. (2008), Use of a Three-Dimensional Detailed Modeling Approach for Predicting Root Water Uptake. *Vadose Zone Journal*, 7: 1079-1088. <https://doi.org/10.2136/vzj2007.0115>
- Landsberg, J. J., and N. D. Fowkes. « Water Movement Through Plant Roots ». *Annals of Botany* 42, n° 3 (1978): 493-508.