

Referee#2:

Dear Referee#2,

Thank you very much for your constructive comments and advice that will help to improve the manuscript and the study. Please kindly follow the point-to-point responses (in blue color) to your comments (in black color) below. Since the revised MS was not prepared at this stage, the referred line numbers here were from the originally submitted MS. All detailed changes and revision were listed here.

On behalf of the authors

Thuy Nguyen

Review of the paper « **Responses of field-grown maize to different soil types, water regimes, and contrasting vapor pressure deficit** »

The paper reports the results of two experiments in which a maize genotype is grown under two soil types, i.e. a stony soil type and a silty soil type, and under two water treatment, i.e. a rainfed treatment that suffers water stress and a fully irrigated treatment. The authors then perform a number of measurement on the stomatal conductance, transpiration, sap flow rate, and assimilation rate, together with a number of water potential measurements at different levels. They also measured the root:shoot ratio in this factorial. One of the important results is the large variation between soil, but also between water treatment, in the root:shoot ratio. This has consequences for the hydraulic conductance of the soil-root-leaf-atmosphere continuum, where the main result is the demonstration that root system and soil-plant hydraulic conductance depended strongly on the soil texture. This is an important result that confirms other recent findings.

The results of the paper are very important, especially in showing the important role of the soil in shaping up plant physiological responses related to the plant hydraulic properties, and as such need to be published.

Many thanks for your supportive summary and comments on the data, results and findings of the study.

That said, I find that the paper is quite long and with too many figures (14). It has a discussion that I think is too long (11 pages) and contains complex elements of the results and does not make enough effort of summarizing, part by part, what are the key messages that need to come up. Therefore, I think the papers needs to be made more concise, may be less technical in some parts of it, before it can be published. It gives at times the impression that it was written as a long report about a series of results, lacking in focus sometimes. As it stands, it does not pay justice to the results that are presented and to the work that was done.

Thank you very much for your constructive and helpful comments. As suggested, we shortened the discussion and removed the information which was repeated from the result section e.g. see the response to comments on L435-478 (shorten the section of root discussion). Also, we have revised the text and added the key messages. Please see the responses to your comments on L389-399 (improvement and clarification in text for Figure 11), L482-489 (add interpretation and conclusion), and L482-522 (add conclusion). These revisions make the MS more concise and less technical in the mentioned sections. Since the combination of Figure 4, 5 and 6 will result in a large Figure, we still keep the number of figures as they are. This will compromise with the comment from Reviewer#1 who supported to keep 14 figures (also supplementary materials) which were informative and relevant to draw the conclusion.

Minor comments:

Thank you very much for your detailed comments and suggestions. We responded to your comments as following:

L108: Replace 'that' by 'because they'

This is done. We replaced with these suggested words.

L117: Space

This is done. See also comments from Referee#1

L128: Insert '.' After 'conductance'

This is done. Thank you.

L134: replace 'it' by 'its'

This is done. See also the comment from Referee#1.

L138: replace 'includes' by 'including'

This is done.

L146-147: may be replace 'different components (root, stem and whole soil-plant hydraulic conductance)' by 'different components of the hydraulic conductance (root, stem, whole soil-plant)'

Thank you for the suggestion. We replaced.

L155-157: Does each of these "rhizotrone facilities" represent what you call later "a site"??

We now consistently used the term "rhizotrone facilities" and removed the "site". More specifically we changed it in the line 158, line 175, and line 478.

L187: replace 'form' by 'from'

This is done. See also comment from Referee#1.

L198: Do you mean to say that over the 7m length of each of these 54 horizontal tubes, 20 measurements (images) were taken in each of them??

Yes. This was what we mean. In total, we have 20×2 (left and right sides) = 40 images from one tube. We rephrased the sentence. Here is the new sentence: "Root images were taken at 20 fixed positions from the left- and right-hand sides of each tube weekly (or biweekly) during the growing seasons."

L206: replace 'of' by 'in'

This was revised.

L210-211: Do you mean to say that you harvested five subplots, each of a 1m², in each replication?? If that is the case, how did you treat the data? You averaged them?

At harvest, we were able to collect biomass samples with 5 replications (each of 1 m²). As the aboveground dry matter data was shown in Figure 14 and in Supplementary material Figures 3a and 3b, the point at harvest was the average of 5 replications. We added one short sentence for the caption of each figure to clarify how we treated the dry matter data at harvest. Now as: “For aboveground dry matter, each point represents the average of two sampling replicates, except the harvest with 5 sampling replicates.”

L304-305: The question is whether you measured such root cracks. And also what was the size of these putative cracks as, if too large, roots may not be able to grow through such air-filled gaps. So, may be clarify whether you had any kind of indication of such cracks, and about their size (in Vertisol you could have cracks that would be 10 cm wide and surely no roots would go through that, so, it is important to know more about soil cracks in that silty soil).

Thank you very much for your suggestion. Your suggestion and concerns are also relevant to the comments from Community (Community comments – Dr. Oliver Dilly) on this manuscript regarding the definition of the soil types. You are right that with the special soils like Vertisols, the cracks could be up to 10 cm wide and roots will suffer from air-filled gaps. For the field in our study, the dominant soil is a Haplic Luvisol that contains a layer with clay accumulation (silty loam texture) (Weihermüller et al., 2007). The thickness of the silty loam layer varies strongly along the slope of the field. It is up to 3 m thick at the bottom of the slope and not present at the top. One rhizotron facility is located at the top (stony) and has a stone content of >60%. The second rhizotron facility is located at the bottom of slope. It has a thick layer with silty loam and a negligible stone content (<4%) and it is characterized by deep soil cracks when dry. However, the cracks were not large (< 5 mm wide) (Morandage et al., 2021). Thus, with this size of the cracks, roots were still able to grow.

References:

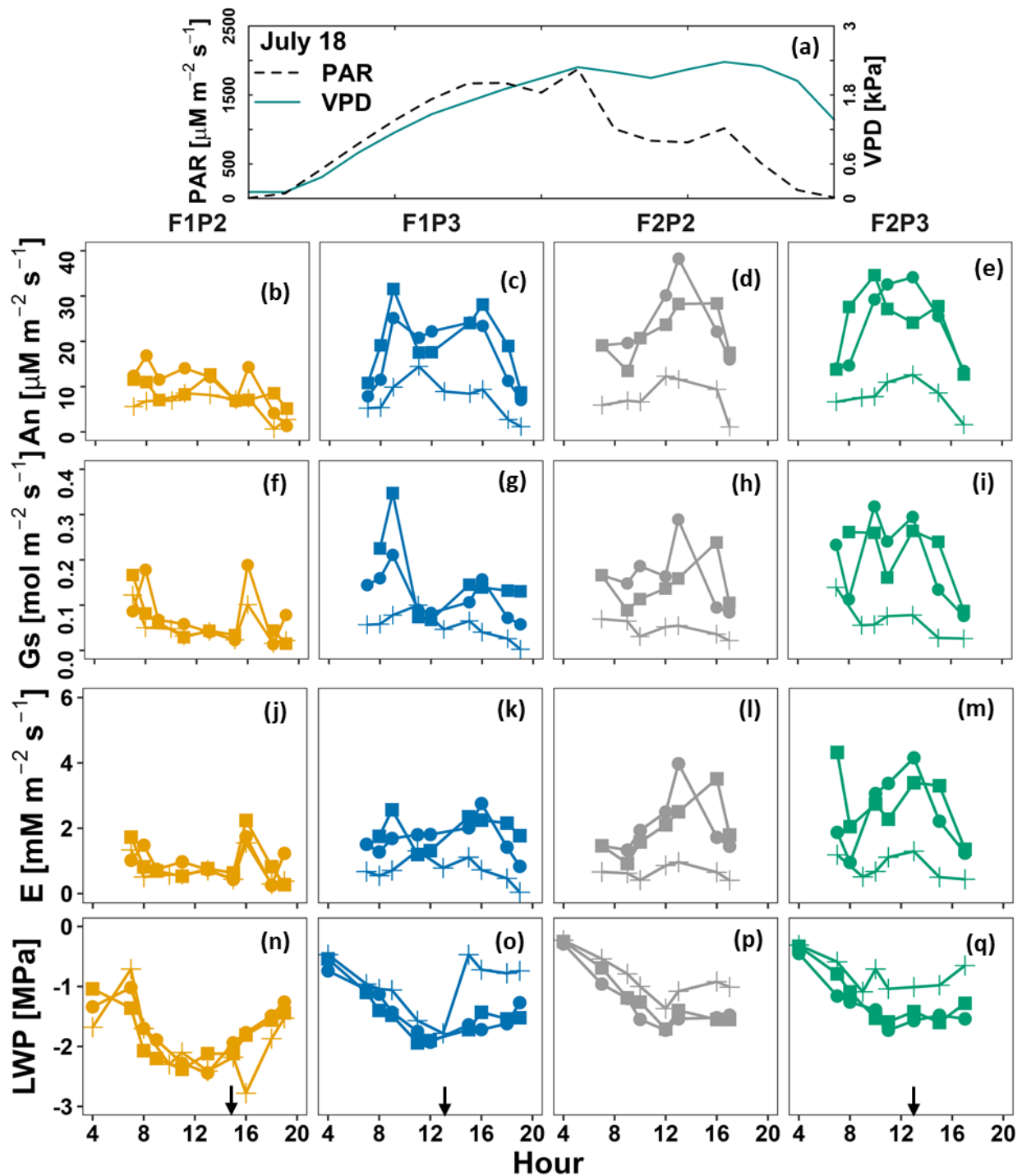
Morandage, S., J. Vanderborght, M. Zörner, G. Cai, D. Leitner, et al. 2021. Root architecture development in stony soils. *Vadose Zo. J.* (April): 1–17. doi: 10.1002/vzj2.20133.

Weihermüller, L., Huisman, J. A., Lambot, S., Herbst, M., & Vereecken, H. (2007). Mapping the spatial variation of soil water content at the field scale with different ground penetrating radar techniques. *Journal of Hydrology*, 340, 205–216. <https://doi.org/10.1016/j.jhydrol.2007.04.013>

L312-328 and Figs 4-6: I wonder if you should group the three figures by showing the continuation of these 4 types of measurements on the same graph, indicating when irrigation would begin and stop. It would much better allow to compare different days, and compare soil and soil-by-water treatment combinations among them for these different factors. The way the figures are laid out and given the relatively small differences it is a bit difficult to do that.

Thank you very much for your suggestion. We understood your point that putting three figures together in one graph will enhance the comparison for different days, before and after irrigation. However, we think combination of all figures (17 sub-plots showing hourly values of 4 main variables – LWP, E, Gs, and An with PAR and VPD for two soil types and two water treatments) could end up with a large and complex figure. Thus, we would like to keep the figures as they are now. However, we added black arrows which indicate when irrigation was applied (e.g. Figure 5n, 5o and 5q). Moreover, the time of irrigation is now mentioned in the text and the captions. We hope the three separated figures with the high quality of resolution and medium information will convey the differences of An, Gs, LWP, and E for different measurement days.

Fig5



L377-378: Here you switch to DOY, whereas in earlier figures you used the calendar dates. This may be a detail but the data presented are sufficiently complex that it would be good to harmonize what can be. So either choose DOY or calendar dates but stick to it.

Thank you very much for your comment and suggestion. We now use consistently the calendar dates in the text in describing the results and discussion that were related to Figure 3 and Figure 10. More specifically:

Line 302 (with Figure 3): add date, now as “Moreover, total root length was relatively equal among treatments at the start of stem elongation (8 June - DOY 159) in both years, while this was the opposite for the ratio of root length to shoot dry matter.”

Line 375-387 (with Figure 10): use date, now as “The slope of linear relationship between sap flow and difference of $\psi_{\text{soil_effec}}$ and $\psi_{\text{sunlitleaf}}$ is shown for three consecutive days (leaf water potential measurements from the predawn) and before and after irrigation applications (17, 18, and 19 July 2018) (Figure 10). On both dates 17 and 18 July, the difference between $\psi_{\text{soil_effec}}$ and $\psi_{\text{sunlitleaf}}$ was around -1.6 MPa with very low transpiration rates in the treatment F1P2 which was associated with very low plant hydraulic conductance and leaf curling. The whole plant hydraulic conductance was disrupted on these two days (0.06 and 0.16 mm h⁻¹ MPa⁻¹ for 17 and 18 July, respectively). Water was supplied on 18 July at 1 PM for the irrigated plots (F1P3, F2P3) as well as F1P2 at 4 PM (for saving plant from death due to severe drought stress). $K_{\text{soil_plant}}$ was slightly changed (0.43 and 0.57 mm h⁻¹ MPa⁻¹ for F1P3 on 18 and 19 July, respectively and 0.5 and 0.58 mm h⁻¹ MPa⁻¹ for F2P3 on 18 and 19 July, respectively). However, the increase of $K_{\text{soil_plant}}$ was substantial in the F1P2 after the irrigation. Soil water replenishment and an increase in the root - soil contact (Fig. 9a) allowed the $K_{\text{soil_plant}}$ to recover overnight to 0.46 mm h⁻¹ MPa⁻¹. This resulted in a narrower water potential gradient between root zone and sunlit leaf and in a higher transpiration rate on 19 July.”

Line 463-464 (with Figure 3): add date, now as: “A slightly higher root: shoot ratio in the F1P2 treatment compared to F1P3 (13 July - DOY 194 & 12 September – DOY 255) was observed in 2017 while the root: shoot ratio in the two treatments was almost the same on 18 July - DOY 199 and 16 August – DOY 228 in 2018 (Fig. 3).”

Line 588 (with Figure 10): add date, now as “The $K_{\text{soil_plant}}$ slightly increased after irrigation (18 July - DOY 199 in Fig. 10b) corresponding with the smaller $\psi_{\text{difference}}$ (Fig. 10b) and an increase in stomatal conductance (Fig. 9c).”

Also, x axis in Figure 8d and Figure 9d will be changed, from “DOY” to “Date”.

Fig8

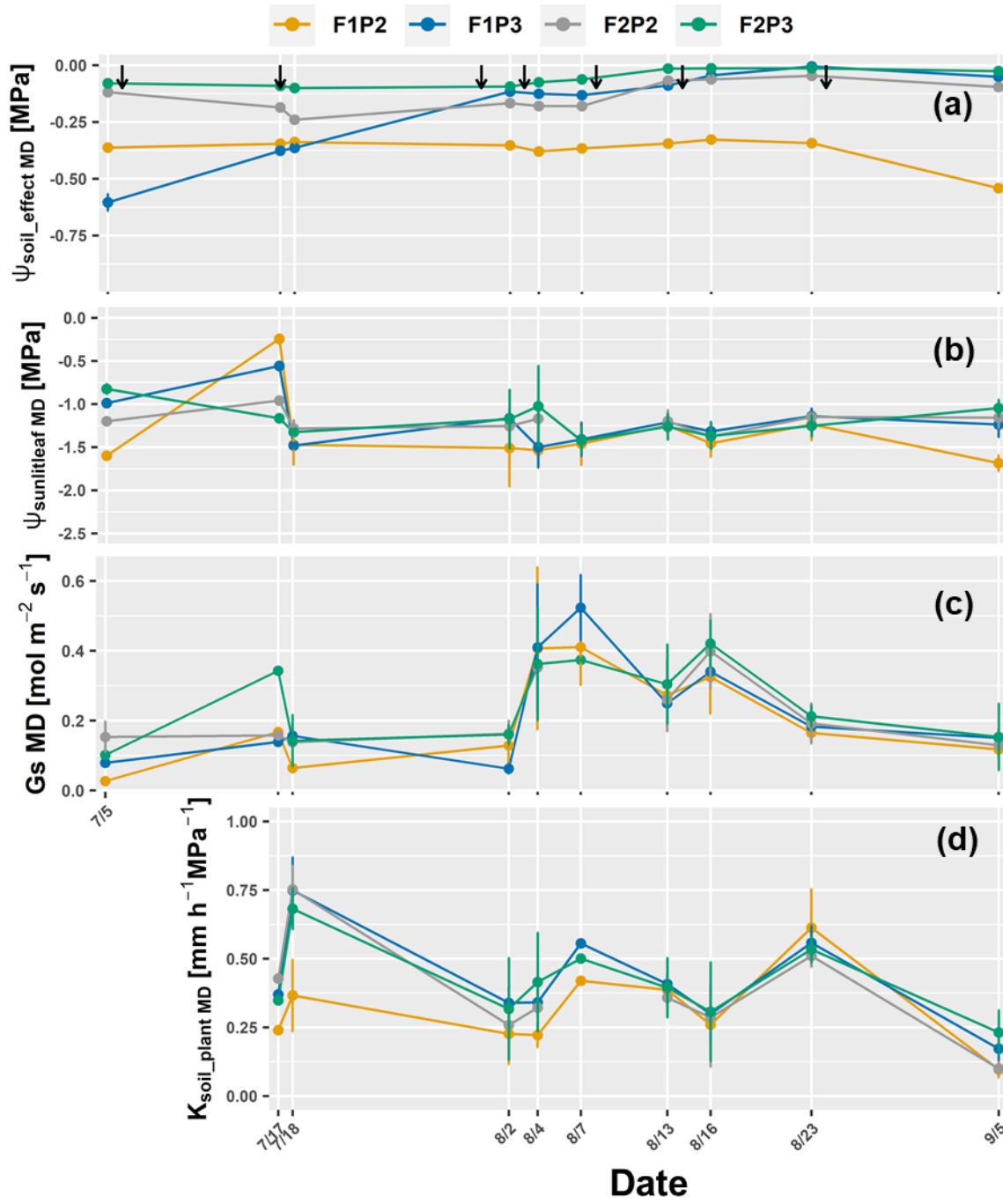
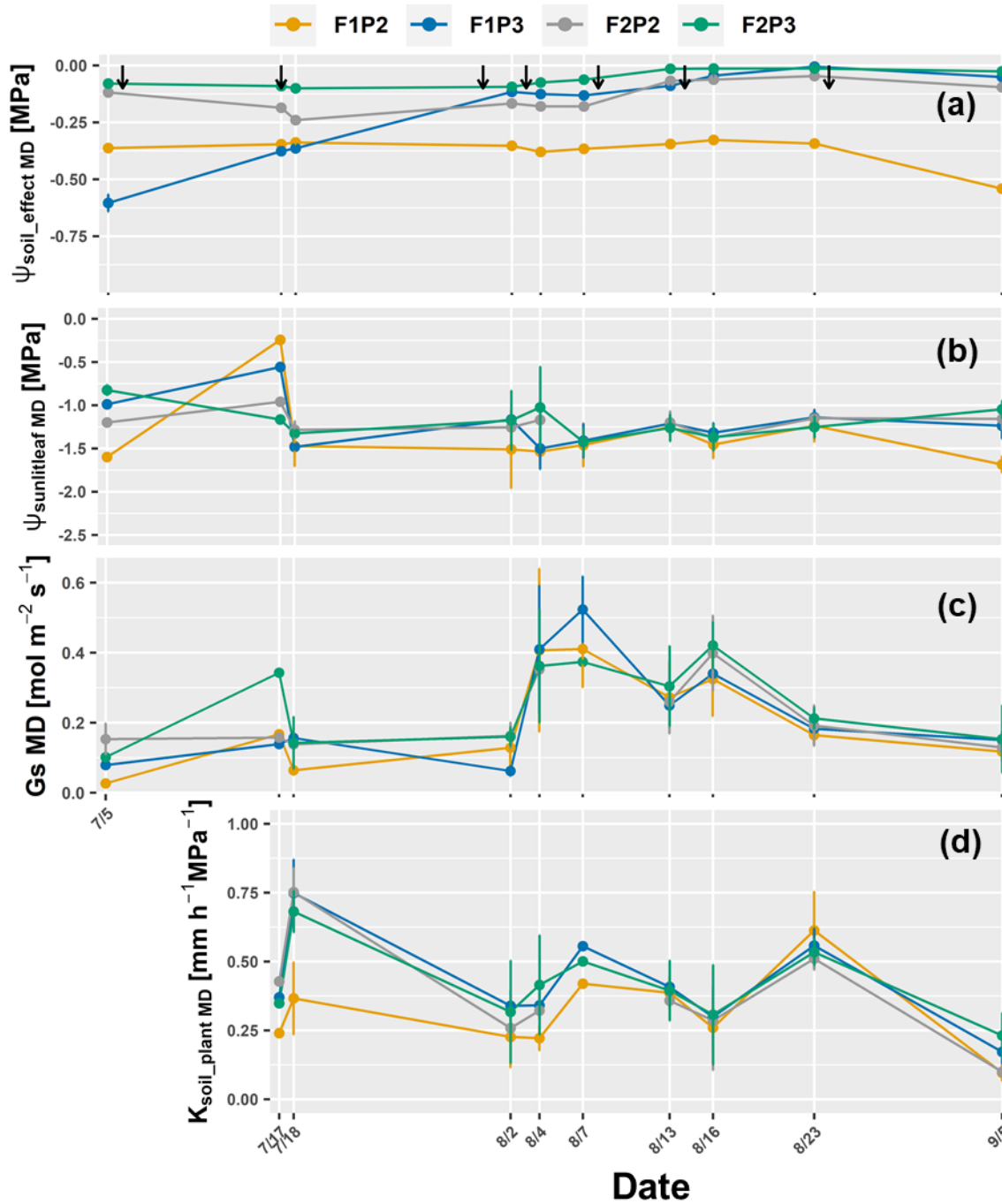


Fig9



L389-399: I see the graph of Fig 11, but the end of the paragraph makes an important interpretation on the data that I have difficulties to follow, possibly because the data that are presented are not adequate for the reader to easily understand that interpretation. Could you clarify?

Many thanks for your suggestion. We revised the text within and at the end of the mentioned paragraph to make them clearer. Now as:

“Seasonal average of different midday hydraulic conductance components (root system hydraulic conductance - K_{soil_root} , stem hydraulic conductance - K_{stem} , and whole plant hydraulic conductance -

$K_{\text{soil_plant}}$) are shown in Figure 11. In the same year, the K_{stem} was not much different among F1P3, F2P2, and F2P3 plots. The K_{stem} of those plots was slightly higher than in the F1P2 in both years. In general, the $K_{\text{soil_root}}$ was lower than the K_{stem} . Overall, the estimated $K_{\text{soil_plant}}$ was around $1 / (1/K_{\text{soil_root}} + 1/K_{\text{stem}})$ regardless of soil types, years, and water treatments. The $K_{\text{soil_root}}$ and $K_{\text{soil_plant}}$ in the F1P2 in 2018 was much lower than the remaining plots while the $K_{\text{soil_root}}$ and $K_{\text{soil_plant}}$ were not much different among plots in 2017. Our results indicated that there was an impact of soil hydraulic conductance on $K_{\text{soil_root}}$ and $K_{\text{soil_plant}}$. Although there is a large difference in total root length between the two soil types (e.g. F1P3 versus F2P2 or F2P3 versus F2P2), $K_{\text{soil_root}}$ and $K_{\text{soil_plant}}$ in those two plots were not much different. This could be explained by the fact that $K_{\text{soil_plant}}$ was not only depended on root length but also depended on the variability of root segment hydraulic conductance.”

L440: Instead of saying that rooting depth was sensitive to the presence of crack, it would be clearer to say if presence of cracks increased/decreased rooting depth.

Many thanks to your suggestion. We revised the sentence. Now as: “Also, both simulations and observations indicated that rooting depth was increased due to the presence of cracks in the lower minirhizontron facility (Morandage et al., 2021) which could explain the high root length between 40 and 120 cm soil depths which was observed in the silty soil in both years.”

L455: you mention root:shoot ratio but then talk about 200-1000 cmg-1, which is a density and not a ratio, please correct.

Thank you for your suggestion. We have used the term: “ratios of root length to shoot biomass” and have corrected the term throughout the text, as in the lines below:

Line 455: now as: “In terms of the ratios of root length to shoot biomass, our observations were in line with those reported in the same soil types for wheat in Cai et al., (2018)”.

Line 458: now as: “Jorda et al., (2022) reported a wide range of ratios of root length to shoot biomass from 200 to 1000 cm g⁻¹ around flowering time of maize depending on the wild type and root hair mutant genotypes growing on either loamy or sandy soils.”

Line 463: now as “We only observed much higher ratios of root length to shoot biomass in the rainfed plot (F2P2) as compared to the irrigated plot on the silty soil (F2P3).

Line 474: now as “The larger ratios of root length to shoot biomass in this F2P2 plot in 2018 as compared to F2P3 could be explained by the change of source: sink relations where more assimilates were devoted to root growth, even at a later growth stage.”

Line 655-656 (in Conclusion): now as “Our results confirmed that root length and ratios of root length to shoot biomass were modulated by soil types and water treatment but less by seasonal evaporative demand. Increase ratio of root length to shoot biomass has been an important response of maize that allows plants to extract more water under drought stress that occurred rather in the silty soil but less in the stony soil due to the higher content of stony material.”

L435-478: This part of the discussion is too long, repeats a number of result part, and then brings elements of literature in a rather scattered and unrelated way (for instance talking about the soil nutrient effects on root length). And finally, it does not give a tangible conclusion of what we should retain about these root length differences between soil texture and between water treatments.

Many thanks for your suggestion. We revised and shortened the text by removing the repetition of result part (Line 463-466) and the soil nutrient effects on root length (Line 444-448), and ratio of root to leaf area (Line 466-469). Also, we removed the comparison of wheat (from previous studies, line 445-447 and line 452-456) and maize (our study) to make the discussion more focused on maize under different soils. In total, **we removed 17 sentences**. As suggested, we added one short sentence at the end of this section to conclude the root length differences were due to soil texture and soil water availability.

The original L435-478 is now much shorter, as:

“Our root observations showed that soil type considerably affected root growth more than water treatment (Figure 2). Root growth was strongly inhibited by the stony soil where much lower root length was observed than in the silty soil, especially in the deeper soil layers. This was consistent with the findings reported in (Morandage et al., 2021) where a linear increase of stone content resulted in a linear decrease of rooting depth across all stone contents and developmental stages. Also, both simulations and observations indicated that rooting depth was increased to the presence of cracks in the lower minirhizontron facility (Morandage et al., 2021) which could explain the high root length between 40 and 120 cm soil depths which was observed in the silty soil in both years.

In terms of the ratios of root length to shoot biomass, Ordóñez et al., (2020) has reported much larger figures of for instance 880 cm g^{-1} in different locations and under different N application rates in maize growing in the Midwest of US. Jorda et al., (2022) reported a wide range of ratios of root length to shoot biomass from 200 to 1000 cm g^{-1} around flowering time of maize depending on the wild type and root hair mutant genotypes growing on either loamy or sandy soils. More roots and higher ratios of root length to shoot biomass were found in the sand than in the loam in both wild type and root hair mutant genotypes (Jorda et al., 2022; Vetterlein et al., 2022). Cai et al., (2018) observed much larger ratios of root length to shoot biomass in drought stressed plots than in irrigated plot in both soil types in winter wheat which indicated the alternation of sink: source relationships to cope with water stress. This study emphasized that more assimilates are used to promote root growth and extract more water under drought stress. However, this was not the case for the stony soil in our work where the drought stress was more pronounced, especially in 2018. A drop of soil water potential (Supplementary material 2b), thus effective soil water potential (Figure 8a) was substantial from 10th July 2018 toward the harvest in the rainfed plot in the silty soil (F2P2) which was consistent with the reduction of leaf water potential (Fig. 8b), leaf area (Supplementary material 3c), total dry matter (Supplementary material 3d), and crop height (Supplementary material 4b) as compared the irrigated plot (F2P3). This indicates a mild water stress in 2018 in the rainfed plots on the silty soil. The larger ratios of root length to shoot biomass in this F2P2 plot in 2018 as compared to F2P3 could be explained by the change of source: sink relations where more assimilates were devoted to root growth, even at a later growth stage. Moreover, the low stone content and soil cracks (Morandage et al., 2021) might favor root growth in the deeper soil layers which are close to the shallow soil water table in the rhizotron facility with silty soil (Vanderborght et al., 2010). In conclusion, both soil texture and water conditions influenced the root growth, however, effects of the former on root length was more pronounced than the latter.”

L481: as affected by?

Thank you. We corrected the heading 4.2.1. Now as: “Leaf water potential and stomatal conductance as affected by soil water conditions”

L484: replace ‘lower’ by ‘less’

We revised the sentence as suggested. Now as: “Moreover, the low stone content and soil cracks (Morandage et al., 2021) might favor root growth in the deeper soil layers which are close to the shallow soil water table in the rhizotrone facility with silty soil (Vanderborght et al., 2010).”

L482-489: you comment your results, then earlier results from others. And these are different. But you leave the reader hanging here: what is your interpretation/explanation for that?

Thank you. We revised the text: specifically moving the first sentence to the end of paragraph then adding one sentence for the explanation of impacts of soil on leaf water potential and stomatal conductance for the previous sentence. This will help the reader to have a clear message. The L482-489 now as:

“In the previous work, Koehler et al., (2022) reported that maize stomata closed at lower negative leaf water potentials in sand than in loam growing under controlled environment. Cai et al., (2022b) investigated transpiration response of pot-grown maize in two contrasting soil textures (sand and loam) and exposed to two consecutive VPD levels (1.8 and 2.8 kPa). Transpiration rate decreased at less negative soil matric potential in sand than in loam at both VPD levels. In sand, high VPD generated a steeper drop in stomatal conductance with decreasing leaf water potential which indicated that the transpiration and stomatal responses depend on soil hydraulics. In our study, stomata closed earlier and at more negative soil and leaf water potentials in the stony soil than in the silty soil (see Fig. 4, 5, 6 and 7). The lower soil water holding capacity of the stony soil compared to the silty soil resulted in lower soil water potential and smaller total plant hydraulic conductance which in turn led to earlier stomatal closure and to more negative soil water potential in the stony soil.”

L482-522: Here also is a fairly long piece of complex discussion that would need to end with a kind of message. I guess the message is that while Welcker and other have show genotypic differences earlier, here you show the soil influence on minimum psi Leaf.

Many thanks for your comment and suggestion. As suggested, we add one short sentence to conclude this key message. Now as: “In conclusion, our results confirmed that the minimum ψ_{leaf} not only depended on genotypic differences but also was influenced by soil types and soil hydraulic conductance.”

L525: Stomatal conductance?

This was revised.

L528: replace ‘estimate’ by ‘estimation’

This was done. Thanks.

L533-539: Here I think it would be better to stick to the units of mm h⁻¹ MPa⁻¹ and then convert the values in 10⁻⁵ h⁻¹ from the citations into that mm h⁻¹ MPa⁻¹ unit which has been used in your graphs.

Thanks. We changed the unit in 10⁻⁵ h⁻¹ into mm h⁻¹ MPa⁻¹ throughout the text from L533-539.

L540-541: That sentence is not understandable

We removed the L540-543 since we think it is also not relevant for the context.

L649: contrasting

It was revised.

L666: and decreased at more negative...

It was revised. Now as: "The stomata conductance was smaller and decreased at more negative leaf water potentials in stony soil than in silty soil."

L667: remove 'plant'

Many thanks. The redundant word was removed.

L668: replace 'that' by 'and'

This was done. See also comment from Referre#1.