Three-dimensional Time-lapse Analysis Reveals Multiscale Relationships in Maize Root Systems with Contrasting Architectures

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Review timeline:

TPC2019-00015-RA	Submission received:	January 7, 2019
	1st Decision:	March 31, 2019 *revision requested*
TPC2019-00015-RAR1	1st Revision received:	April 19, 2019
	2nd Decision:	April 30, 2019 *accept with minor revision*
TPC2019-00015-RAR2	2nd Revision received:	May 8, 2019
	3rd Decision:	May 12, 2019 *acceptance pending, sent to science editor*
	Final acceptance:	May 23, 2019
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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2019-00015-RA   1st Editorial decision – *revision requested* **March 31, 2019**

As you will see from the reviewers' comments below, their main concerns involve the availability of the raw data, statistical analyses and related data analysis. The reviewer's question whether claims on the persistence of traits and quantitative statements related to Figure 4 is justified based on the current analyses. Please address these issues and all other points raised by the reviewers when revising your manuscript.

-- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2019-00015-RAR1   1st Revision received **April 19, 2019**

Reviewer comments and **author responses**:

Reviewer #1:

The manuscript entitled, "3D time lapse analysis reveals multiscale relationships in contrasting maize root architectures" used optical reconstruction of seedlings grown in gel, and X-ray imaging of root crowns excavated from the field, to demonstrate potential differences in allocation to the root system in seedlings that potentially explain differences in the field as well. The optical imaging platform in gel allowed relatively easier root segmentation than X-ray or MRI, which let the authors focus on the temporal aspect, which is generally neglected, even in non-destructive root imaging. The authors used an elegant solution to align the same root systems over time and determine growth patterns. Overall, seedling root system mass did not change, but the pattern of allocation did, potentially underlining the statement that investment in cheaper root classes allows greater total root length and potentially nutrient and water uptake from soil.

Overall, I found the manuscript well written, providing insight into processes that are difficult to study, and with reasonable conclusions and interpretation. In addition, I only have the following line comments:

Point 1. Broadly, the seedling differences in allocation to axial versus lateral roots matched the results from X-ray field root crowns. Of course, this suggests that the seedling analysis predicted the results obtained in the field, but the authors should acknowledge in the text that this is too small a sample size to be conclusive.

RESPONSE: We agree. We added this statement directly in Lines 251-255 "Given our small sample size and analysis of only three maize genotypes, these observations are not meant to be conclusive, but to underscore the influence of endogenous developmental mechanisms on RSA at any scale, and to provide an example of how emerging high-information content root phenotyping tools can be used to re-evaluate conventional wisdom."

We also added the conditional "can" or "could" in front of statements about persistence or prediction, for example in the Abstract Lines 28-29: "3D imaging of mature field-grown root crowns, the nexus of the root system, showed that several genetic differences in seedling architectures *could* persist throughout development and across environments"

Point 2. Line 41: The term root system architecture (RSA) is more accurate than root architecture, and I suggest using it throughout the manuscript.

RESPONSE: We changed every instance to either "root system architecture" or "RSA", including the Title, with the exception of the Short Title because it violated the word limit.

Point 3. Line 242: With only 3 lines, it is difficult to make a meaningful claim about the relationship between artificial and field environments that could be a coincidence. This should be acknowledged.

RESPONSE: We believe we have adequately acknowledged this concern in the aforementioned reference in Lines 251-255.

Point 4. Line 407: In agriculture, we'd like plants to neither compete with themselves nor their neighbors, but rather optimize soil exploration at the stand level.

RESPONSE: Good point – we clarified this in Lines 423-426: "In this context, plants in the wild explore the soil while competing against other root systems for resources, or in the case of agriculture, to optimize resource capture at the plot level."

Point 5. Line 475: SO after reading about plant materials, growth, and imaging - I don't understand how you got the time lapses for each genotype. Looks like there was a total of 38 plants. Were they all grown simultaneously? How were they each measured, is there a conveyor or something, or do you have 38 imaging systems?

RESPONSE: Unfortunately, our process was not nearly so sophisticated or high-throughput! We measured each plant one-at-a-time. We clarified in Lines 493-495: "Due to the nearly real-time imaging frequency, plants were imaged one-at-a-time during a week of growth, before the next plant was loaded into the system."

We note that our analyses in S4-S6 suggest we could get largely the same model parameter value difference with 24h time intervals (rather than 4h), which would allow us to conduct the time series experiment manually, but in much higher throughput, at least 50+ per week with our current system.

Reviewer #2:

Point 1. It is good practice to publish data along with the manuscript and the source code to ensure reproducibility of the results. In this manuscript, the 3D data is missing and the provided link to the GitHub repository is not valid any more.

RESPONSE: We absolutely agree. The GitHub link did not work because we neglected to convert the private repository to a public one. This has been rectified and the link should work now. We have provided all code, the 3D root models, the Dynamic Roots outputs, and the raw data from extracted features. This information can be found in the *Methods: Data and software availability* section Lines: 577-584.

Point 2. Overstatement in Line 213: Seedling root architecture traits persisted in field-grown mature root systems. The presented PCA does not support this claim. If the authors want to show that traits persist , a correlation analysis with r^2 and RSME between traits would be more appropriate. The current PCA only concludes that the mean persists in a certain projection of a higher dimensional space. Visually, the PCA plot does not support the conclusion of similar variance or other higher order statistics.

> **RESPONSE: We see your point. The original analysis was focused more on the fact that when we measured a significant difference between B73 and Mo17 in the seedling assay, we would see the same *relative* difference in the field data. This was true for all features we measured except convex hull, which is strongly affected by the sampling method in the field (Figure S3). The PCA in Figure 2 is meant to portray this overall trend, and we are now more explicit about this in Lines 231-234: "In multivariate space, our analysis clearly delineated the two inbred genotypes, *rather than growth stage or environment*, with the first two principal components (PCs) explaining ~75% of the total phenotypic variation in the combined data set…"**

> **But more to the point, we now directly compare the standardized trait distributions for a given genotype in the gel or field in Table S6. These results are described in Lines 237-244: "Although we could not directly correlate gel and field values due to our unbalanced data set, we compared the standardized distributions for each trait in both environments and found that solidity (a measure of the thoroughness of exploration), specific root length (a measure of root biomass allocation to relatively thinner or thicker roots), the total root length and total number of root tips persisted across growth stages and environments for all genotypes, while the persistence of convex hull volume and width to depth ratios were conditional on genotype and was not supported for root system volumes (Figure S3; Table S6)."**

> **So now we believe our statements of persistence are validated, although somewhat more nuanced. Partially in response to Reviewer #1's concerns about small sample size and also our new analysis, we added conditions to our statements of "persistence" such as in Abstract Lines 28-30: "3D imaging of mature field-grown root crowns, the nexus of the root system, showed that *several* genetic differences in seedling architectures *could* persist throughout development and across environments" and in the topic heading in Lines 214-215 (previously 213) which now reads "Seedling root system architecture traits *can* persist in field-grown mature root systems".**

Point 3. Figure 4 is very central to the manuscript but lacks quantitative description! Currently it is simply a visual comparison. Differences between distributions and their significance would strengthen the argument made. In particular, the following derived hypothesis that states that a global growth pattern exists would profit from the qualitative underpinning of Fig. 4 and explanation of the results in the text. Currently, the existence of a global growth pattern comes across as an overstatement. As a personal note, I do agree with the hypothesis, but the quantification that leads to the hypothesis is missing.

> **RESPONSE: We were too qualitatively descriptive of the trends and should have been more rigorous. We have now conducted Kolmogorov-Smirnov tests to test for differences in the various trait distributions (Table S8-S10), and modified the text to provide this quantitative support to our description of the trends in Lines 310-347. Our conclusions are largely the same, except we now find the soil angles between B73 and Mo17 in the first 48 hours to be similar, whereas by eye we thought B73 was steeper, Lines 333-335.**

Point 4. Line 90-92: "But (for many practical reasons) the vast majority artificially constrain root growth to two dimensions, adding risky assumptions to 3D modelling applications." This reads odd and a bit out of context. I believe the authors tried to explain that constraining root growth to 2D space allows only the observation of the phenotype in the constrained environment. Therefore, inference to understand the gene x environment interaction of the plant in field environments is limited.

> **RESPONSE: That was an awkward sentence! We now revised this in Lines 89-92: "But (for many practical reasons) the vast majority artificially constrain root growth to two dimensions, which limits understanding of the actual 3D phenotype, especially in field environments."**

Point 5. line 533 -535: "Since the features from the two stages had different scales, we standardized the data by removing the mean and dividing the standard deviation for seedling and mature roots separately." This sentence is totally unclear to me. What does this mean? Removing the mean? Dividing the standard deviation? That is

nonsense. Did you want to use the z-score, but didn't knew about it or didn't figure out how to handle it? Something is strange here and simply needs careful explanation.

> **RESPONSE: Yes, this is the Z-score. We were trying to explain this commonly used method for standardizing distributions from different scales in words, but now we simply provide the equation in Lines 558-565.**

TPC2019-00015-RAR1   2nd Editorial decision – *accept with minor revision*   **April 30, 2019**

Editor comments and **author responses**:

Point 1. For clarification, please improve the presentation of the Kolmogorov-Smirnov test. Typically, the D-statistic is reported and discussed along with the corresponding p-values. The KS-test is great to show differences and similarities between distributions in this case. However, it is not OK to only tell half of the story, especially if the audience is predominantly experimentalists. KS is not a test that is taught in the biology curriculum too often due to its sensitivity issues (sometimes one has to use the Anderson-Darling test instead). Hence, you need to explain to the reader why distributions are deemed to be similar or dissimilar. How you choose the right D and p to come to this conclusion is important to know. An appropriate visualization of this test is the histogram or cumulative frequency histogram for each distribution with both on the same plot with the same x-axis. Please update Figure 4. All conclusions of the paper build upon Fig. 4 and its derivatives.

> **RESPONSE: We agree that our initial presentation of the KS-test was not adequate, and have made several changes in this revision. Most notably, we now report the D-statistic and calculate its critical value (D-critical, explained in Methods Lines 584-590), which we apply as our threshold of similarity or dissimilarity. We modified Figure 4 to include visual information about whether D-critical was reached at each time point, for each genotypic comparison, for each of the three traits we analyzed, in a way that should help the reader understand our interpretations of the similarities or differences in growth patterns of the different genotypes.**
>
> **We added this text to explain Lines 312-314: "For each genotypic comparison at each time point we computed the critical value of the D-statistic (D-critical; Methods), above which we conclude the distributions differ between genotypes and vice-versa (Figure 4)." Additionally, we removed the previous tables summarizing the p-values of the KS-tests and replaced them with panels of cumulative frequency histograms as suggested (Supplementary Figures 10-12), including also the D- and p-statistic values for each comparison.**

Point 2. The table caption in the supplemental material has the same problem. It is unclear if D or p values are given in the table. Nothing is explained and only one of the two values is reported.

> **RESPONSE: We have removed these tables in lieu of the revised Supplementary Figure 10-12, which we believe adequately address these concerns**

TPC2019-00015-RAR2   2nd Revision received   **May 8, 2019**

TPC2019-00015-RAR2   3rd Editorial decision – *acceptance pending*   **May 12, 2019**

We are pleased to inform you that your paper entitled "3D time lapse analysis reveals multiscale relationships in contrasting maize root system architectures" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor   **May 23, 2019**