Field Crops Research

The roots of the rotation effect run deep

--Manuscript Draft--

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Abstract:	Context or problem It is well-established that maize (Zea mays L.) grown in extended rotations produces higher grain yields compared to maize grown in one- or two-phase rotations, even when nitrogen (N) is not limiting. Understanding the mechanisms driving this phenomenon, commonly referred to as 'the rotation effect', is important for designing cropping systems that use land and resources efficiently. Differences in root systems can influence resource acquisition and therefore yield, but it is unknown if such differences play a role in the rotation effect. Research question We hypothesized that maize grown in an extended rotation system exhibits a deeper root structure with less biomass investment compared to maize grown in a short rotation, and that these characteristics would be correlated with differences in grain production. Methods Using a long-term experiment established in 2001, we measured maize rooting depth across the growing season, root biomass in 15 cm increments from 0-60 cm, and grain yields in the maize phase of two contrasting rotations: a 2-year rotation of maize/soybean (Glycine max [L.] Merr) using inorganic sources of nitrogen (N) and maximum tillage depths of 15 cm (hereafter the 'short rotation'), and a 4-year rotation of maize/soybean/oat (Avena sativa L.)-alfalfa (Medicago sativa L.)/alfalfa using a mix of organic and inorganic N sources and inversion tillage to 25 cm (hereafter the 'extended rotation'). Additionally, we measured soil penetration resistance and soil moisture, and performed a growth analysis on aboveground maize biomass. Results From 2013-2020, maize grain yields in the extended rotation were equal to or significantly higher than in the short rotation, averaging 8% higher across eight years (11.0 and 10.2 dry Mg ha-1, respectively). In three seasons (2019-2021), the maximum rooting depth of maize in the extended rotation was significantly deeper than in the short rotation by an average of 11% (82 versus 76 cm, respectively), although the timing (e.g., early season, late season) of the extended rotation's maize growth advantage was not consistent across years. At physiological maturity, the two systems had similar amounts of root biomass from 0-60 cm soil depth, but maize grown in the extended rotation invested significantly less of that biomass (30% compared to 47%) into the soil surface layer (0-15 cm). The soil penetration resistances of the two systems differed in a manner consistent with the differing tillage regimes of the two rotations, however the patterns did not align with root differences. Conclusions We posit that the extended rotation's 'deeper and steeper' maize roots did not guarantee higher maize yields, but rather bestowed the plant with more flexibility in resource acquisition which, in certain conditions, resulted in higher grain yields compared to maize grown in the short rotation. Implications

	To our knowledge, this is the first report attempting to mechanistically link rooting patterns with plant growth in the context of the 'rotation effect.' This study enhances our understanding of how cropping system histories impact yields, and provides new data on yields and roots, both of which are highly relevant for sustainable intensification. While the present study focused on physical measurements, it suggests that more detailed exploration of how biological drivers impact root architecture is needed to gain a mechanistic understanding of the 'rotation effect.'
Suggested Reviewers:	Amelie Gaudin, PhD Associate Professor, University of California Davis agaudin@ucdavis.edu Dr. Gaudin has worked with maize and diverse crop rotations throughout her career.
	Michel Cavigelli, PhD Soil Scientist, United States Department of Agriculture Baltimore Field Station michel.cavigelli@usda.gov Dr. Cavigelli has worked on crop rotations for much of his career.
	Gregg Sanford, PhD Associate Scientist, University of Wisconsin-Madison gsanford@wisc.edu Dr. Sanford has worked with a long-term cropping systems study in Wisconsin and would have good perspectives to offer regarding this study.
Response to Reviewers:	Reviewer #1: Summary: Maize can be grown in a continuous maize system, in a simple rotated maize system, or in an extended maize rotation. Previous research has shown an increase in yield in the extended maize rotation compared to a simple rotated maize system. Differences in resource distribution, microbial communities, and nutrient cycling have also been observed between these two systems. The authors investigate how the extended rotation influences maize root growth and its implications on yield. Over the investigated time period, maize yield is shown to be on average higher in the complex/extended rotation system compared to the simple rotation. This increase in yield also corresponds to an increase in rooting depth, less root biomass reported in the top 15 cm, lower soil penetration resistance, and lower soil moisture in the complex system compared to the simple rotation. The authors claim that the complex rotation system results in a cheaper and steeper root system which helps increase the likelihood the plant can withstand unfavorable growing conditions. They suggest the changes in soil physical, chemical, and biological properties contribute to this resilience. Major comments: 1. The paper claims that the difference in root growth is due to the increased complexity of the extended rotation. However, it's unclear how the difference in fertilization method (inorganic vs manure) and differences in tillage practices (moldboard plowing after alfalfa and before corn) influence soil physical, chemical, and biological properties. Past studies on the same field acknowledge the possibility that fertilization by manure and moldboard plowing can contribute to differences in soil properties. These factors are mentioned at the end of the discussion, but it would be helpful to mention this in the introduction and in the results when discussing root growth and soil physicochemical properties. We agree, we reworked the introduction and added text to explicitly acknowledge these complexities. 2. Due to the presence of significant root biomass at maize planting, the authors use the difference in root biomass from the beginning and end of the growing season to compare the two systems. This method leads to a negative root biomass in the 0-15 cm soil depth. While I acknowledge roots from a previous growing season could lead to an overestimate of the maize root biomass, the negative value suggests that a significant amount of the alfalfa roots decomposed by maize maturity. It does not seem accurate to take the difference in this case. Also, given that alfalfa has a favorable C:N ratio and the application of composted manure, the alfalfa roots are in very favorable conditions for rapid decomposition. This is a good point, and prompted us to rework our root biomass analyses. We instead look at extreme cases of assumed background root decomposition. In all but the most extreme case (no background root decomposition in the short rotation and 100% background decomposition in the extended rotation) the statistics show the short

rotation maize produced more root biomass than the extended rotation maize in the 0-15 cm depth. In other words, the conclusion does not change, only the magnitude of the difference. This lends confidence that our results are robust.

3. In addition, the negative root mass in the top 15 cm is ~125 kg ha-1 which accounts for a majority of the difference in total root biomass (~180 kg ha-1) between the two systems. Factoring in that there must be root growth in the top 15 cm that connect the root biomass at 15-30 cm soil depth, I would guess there isn't a significant difference in total root biomass.
Indeed, using our new analytical approach we found there was no conclusive evidence that there was a difference in total root biomass between the two cropping systems, only the way it was distributed in the soil. We added text to clarify this finding in the redrafted manuscript.

4. The root biomass addition method is also confounded by the fact that microbial activity decreases with soil depth. The deeper alfalfa roots may not decompose as readily as those in the top 15 cm. So, subtracting out the root biomass at planting at all soil depths may be accurate at deeper depths but not accurate for shallower soil depths.
By using the ‘extreme assumptions’ ranges noted above, we addressed this issue. The differences at the deeper soil depths were sensitive to the assumed decomposition, so we do not claim differences between the cropping systems in root production at those depths (>15 cm).
5. The method for collecting root biomass described by the authors likely has significant error and likely contributes the large error bars in Figure 3. Root biomass is collected from 4 samples collected from 32 mm diameter (1.26 inch) soil corers. This is roughly 32 cm2 (5 square inches) area in total. There is likely an extremely high variability in root biomass especially at lower depths where roots are much sparser and would depend on the ability to capture a root in that segment. Thus, it's hard to compare the systems at depths below 15 cm.
We agree with the reviewer that there may have been true differences between cropping systems at depths below 15 cm that we were unable to detect due to our sampling scheme. We added text to the manuscript to acknowledge this possibility. We note that the coefficients of variation of the measurements showed no pattern with regard to depth, so taking more subsamples in each plot may not have reduced the variation at lower depths, and therefore may not have resulted in more statistical power – it’s difficult to say.

6. Authors suggest the complex system is more resilient to extreme weather events which increases yield. The authors provide broad characterization of the average weather conditions for the measured years (hot vs cold and wet vs. dry). It would be nice to include some measure of the extreme weather events from those years (heavy rainfall, heat waves, droughts, frost, etc.) as these events and their timing may contribute to differences in yield.

We initially tried to parse out relationships between the yield differentials between the two rotations and more nuanced growing condition events, however it very quickly became complicated because ‘extreme’ events may only be extreme relative to the crop stage, and many do not impact fields uniformly (e.g. hail, extreme wind). It was therefore difficult to quantify how ‘favorable’ or ‘unfavorable’ a given year was without coupling the study with a crop model that can express drought, heat, or excess water stress in a quantitative way. In the re-drafted manuscript, we softened our language to avoid suggesting extended crop rotations can buffer against all types of extreme events.

7. The differences in root biomass are only reported for 2019 and 2020 when there is not significant difference in yield. It's unclear whether in a year where there is a greater difference in yield results in root biomass trends still hold true.
This is a good point - we added text and additional data demonstrating these patterns in a year with a significant difference in yield to address this concern.

8. In line 162, the authors describe the complex rotation maize roots as "more functional." And, in line 245, the authors claim the complex rotation achieved a more efficient root system with less resource investment. However, roots respond to

resource availability as noted by the authors in a separate part of the discussion. Previous publications from the same field experiment note differences in POC, microbial activity, and soil physical properties. It would be more appropriate to frame the difference in root system architecture to be a result of differences in soil properties rather than the maize plant investing in steeper and cheaper roots especially since the maize genotype is the same. In the Lazicki et al., they note that differences in root length density seem to correlate with POC content.
This is a good point. In the re-drafting of the manuscript we frame the changes in soil physical, chemical, and biological properties due to cropping systems as creating opportunities for dynamic responses by maize roots, which may be linked to yield enhancement under certain conditions.

Minor comments:
1. Degradation of the alfalfa root biomass will impact N and P availability and likely influences rooting. In addition, the weather conditions will influence microbial activity and litter decomposition rates.
Yes, that's true. To accommodate differences in nutrient addition between cropping systems, N fertilization followed protocols associated with the Late Spring Nitrate Test, and P fertilization followed recommendations based on soil samples drawn from individual plots.

2. In table 3, it's unclear how the timing of maize growth advantage is determined/calculated.
We added a superscript to clarify this – early refers to time periods before the maximum growth rate occurred, and late to time periods after the maximum growth rate occurred.
Also, are the p-value based significances comparing values (ratios and timing) between years? I was confused by how p values were determined for this table.
We added references within the table caption to clarify where the values and significances came from and added text to clarify that the ratios of absolute values are presented only to aid in visual comparisons of the values/patterns.

3. In table 3, please include the values for 2020 and 2019. Even though it is not significant, the root information is mainly for those two years and would help put into context how root growth may impact yield.
We reworked the entire table to include the root data and simplify its visual message.

4. The authors note that soil from the complex system resulted in plants with finer roots when grown in the greenhouse. It would be nice to add in a small discussion about how microbiome composition can influence the plant allocation to coarse vs fine roots. For example, root order has been shown to impact microbiome composition, and AMF have been shown to induce lateral root growth in plants. This may be especially important given that a previous publication focused on these fields (King and Hofmockel) observed higher microbial biomass in the more complex rotation.
We did not quantify differences in coarse versus fine roots in our study, so we chose not to include this in our discussion.

5. Inorganic N is highly mobile in soils, and increased wetness/soil moisture may result in more leaching and less N taken up by the plant in the simple system versus the complex system which has more sustained release through the organic N source (manure).
Yes, see Figure 2 in Tomer and Liebman (2014, http://dx.doi.org/10.1016/j.agee.2014.01.025).

6. There is a difference in the inorganic N applied at V6 between the simple and complex systems. Is this due to differences in soil nitrate measurements at that time? If so, this could point to differences in soil N over the growing season that may influence yield as mentioned in the previous comment.
The difference in applied N at V6 was due to differences in soil NO3-N concentrations and recommendations for side-dressing from the Late-Spring Nitrate Test protocol that were cropping system-specific. We added this to the management table to clarify this.

7. At line 98, the authors note that the difference between system additions was not statistically significant. Even if it is not statistically significant, there is a noticeable

difference as shown in Figure 3, and this trend is consistent with previous root data (root length density measurements from Lazicki et al.) from the same fields. Lazicki et al observe a more even distribution of roots throughout the soil column in the complex system versus the simple system.
We reworked the statistical analysis and in the process addressed this issue. Additionally, we referenced Lazicki et al.'s work more explicitly, adding a supplemental figure summarizing their findings.

8. The higher penetration resistance at depths below 30 cm in the complex system may suggest that the biopores created from deeper alfalfa roots contribute to the differences in rooting depth and the more even distribution of root biomass along the soil profile.
We added this to the discussion. We are unsure whether the biopores would show up in the resistance measurements, so changed the terminology to refer to the penetration resistance of the bulk soil, as we believe that is what is being measured.

Reviewer #2: This study enhances understanding of the 'rotation effect'in agricultural production systems, and provides new data on yields and roots by long term positioning experiment. However, it is unclear what the physiological and ecological mechanisms are, and further supplementary data is needed to support the Conclusions.

Reviewer #3:
This manuscript sets out to show whether changes in root systems explain the rotation effect. It reports many measurements and concludes that chemical and/or biological factors are responsible but physical factors are not. These conclusions at the end of the
Discussion are hard to justify because neither chemical nor biological factors were measured.
We rewrote the results and discussion to clarify the evidence that supports our conclusions.

The strength of the manuscript is the extensive data collection, the mostly complete statistical analysis and clear expression. The weakness is that the control of the rooting depth and its connection with yield are not clearly explained. This comes about because the long-term experiment was not designed to clarify the topics discussed in this manuscript. The authors should make revisions that more closely link these data to yield. The catchy title deserves retention; it is a little deceptive because cause and effect are unclear and this should be clarified in the abstract and conclusions.
We hope the redrafting of the discussion section has led to a clearer proposed link between our data and yield. Additionally we adjusted the language in the abstract to clarify our evidence and the conclusions we draw from it.

The manuscript implies that the reported 'complex rotation system' is representative of complex rotation systems in the Midwest US. Clear evidence for such equivalence should be reported but if it is not available the manuscript should address only the particular four-course rotation system that was studied and not extrapolate the results to other, undefined, 'complex' rotations.
This is a valid concern. We believe we address this in the introduction where we define 'general' extended rotations for the Midwestern region of the US as those including a small grain and/or forage:
Midwestern maize-based systems fall into three main categories: (1) continuous maize systems, wherein maize is grown for two or more consecutive seasons; (2) simple rotated maize systems, wherein maize is rotated with soybean; and (3) extended maize rotations, wherein maize is grown in a rotation with two or more years between maize crops, often including a small grain such as oats (Avena sativa) and/or a forage crop such as alfalfa (Medicago sativa).
This applies strongly to references to papers where it is not clear if the complex rotation is identical to the one reported here.
All citations extend from this definition presented in the manuscript:
The maize yield advantage accrued from extending short rotations to include small grains and forage legumes has received less attention compared to the continuous

maize penalty, but has likewise been well-documented (Liebman et al., 2008; Stanger and Lauer, 2008; Coulter et al., 2011).
While it is almost impossible for a rotation to be identical due to regional differences in markets, soils, etc. all of our 'extended' rotation references refer to maize rotations that include a small grain and/or forage.
Value-neutral names of the systems would be two-course and four-course rotations.
We changed the terminology to 'short' and 'extended'.
Attributing maize yield to a system is less convincing than to a particular part of the system such as crop species or management operation. It is difficult to unscramble such effects from the data but the authors are in the best position to do so.
We rewrote the introduction and parts of the discussion to make it clear why it is difficult to attribute such a complex characteristic such as yield to a single change, when extending rotations involves a slew of changes that cannot be controlled for individually. We try to present the factors that may be contributing, and in reality is unlikely it is one factor.

The emphasis on nitrogen is unbalanced. It appears on the second line of the abstract and in many parts of the manuscript but there are no reported measurements of soil mineral N. This is a matter of concern since there is much published evidence that crops growing after alfalfa benefit from residual N arising from biological N fixation. If there are no such data available for this experiment, are there published or unpublished data from other studies to fill the gap? The N supply to maize in the four-course rotation also includes a large amount of N in manure, as reported in Table 1 but N-supply is not discussed as a possible reason for the higher yield.
We added citations and text to demonstrate these yield enhancements are observed even when nitrogen is not limiting. We also added text to Table 1 to demonstrate how the amounts of N applied to the two systems were balanced by the side-dressing which was based on a late-spring nitrate test. While the forms of nitrogen supply differed, the absolute amounts did not.

Please provide as much information as possible about the oats and alfalfa; were they cut or grazed and is it possible to report the amount of N contained in alfalfa, both above-ground and below-ground.
We added information indicating the management of the oat straw and alfalfa (oat straw and alfalfa hay were harvested, baled, and removed from the research site, no grazing occurred on these plots). We do not have data on the N content of the below-ground alfalfa biomass. Osterholz et al. 2018 does an in-depth exploration of nitrogen pools in these systems and found they could not explain the differences in maize nitrogen dynamics/yield – we added a more explicit citation of that work.

At L107 there is mention of biological and physical effects on root systems but less prominence is given to chemical factors. The nearest that the manuscript comes to reporting chemical effects is the large amount of manure-N. This should be strengthened.
We added mention of ethylene build up and its potential role in root growth. See above comment concerning N.

The shallow rooting depths (Figs 2 and S2) in some seasons is explained by excess soil water. Could the deeper maize roots in the four-course rotation be due to dewatering of the soil profile by the previous alfalfa crop?
The shallow rooting depth in 2018 was due to high water tables. Our soil moisture sensors at 45 cm depths indicate no difference in soil water contents at that depth between the two rotations, and indicates the soil was fully recharged with water following the alfalfa crop termination the previous fall(s). In general, in this region the entire soil profile goes through a resaturation event before spring's maize planting activities.

The discussion of the effect of alfalfa should include mention of hydrogen fertilisation. Presumably there was hydrogen fertilisation by the alfalfa rhizobia but any contribution by soybean rhizobia would depend on whether they were HUP-plus or HUP-minus.
We considered mentioning this in the original manuscript draft, but in the end felt it was a distraction. The soybean inoculant was HUP-minus, so any differences would be a result of the different quantity of roots releasing H_2, and we have no data on the

	soybean nor alfalfa roots in these plots. While certainly and interesting area of research, we opted to omit it in this manuscript. The end of the Discussion suggests that roots in 'more complex' rotations are better buffered against unfavorable growing environments. In Fig. 1 the four-year rotation seems to perform best in the wet seasons of 2016-2018. This apparent inconsistency should be discussed. While the absolute yields of the four-year rotation were highest in 2016 and 2018 as the reviewer points out, we were interested in how the four-year rotation performed RELATIVE to the two-year system. The size of the points on the right panel represents this RELATIVE difference, and it shows that this relative performance was higher in a range of weather conditions (for example in 2013, a hot and dry year, and in 2018 (a hot and wet year). Detail Report the statistical significance of the higher yield, rooting depth, root biomass and penetration resistance in the Abstract and Highlights. Word limits precluded the inclusion of statistical significances in all instances, but we added the significance in several places within the abstract. We hoped that the context would convey that any results reported as being different can be assumed to be significantly so. The Conclusions (L258) that the result is 'novel' is inconsistent with many papers referred to in the manuscript. We adjusted that language to better articulate the exact component of our study we feel is novel. The renumbering of lines makes it difficult to review the manuscript. Please stick to one system in any resubmission. Yes, we apologize, the line numbers restarted after the landscape table which required a section break. We have corrected this issue. The references are reported in several different formats and should be standardised. We corrected the references, switching between citation systems is always a challenge.

February 6, 2024

To the Editorial Board of Field Crops Research:

On behalf of all co-authors, herewith I am submitting a revised manuscript entitled “The roots of the rotation effect run deep” to be considered for publication in Field Crops Research.

Based on the constructive feedback provided by the reviewers, we have made substantial changes to the manuscript, resulting in significant improvements in clarity and quality. We have addressed each reviewer’s comments in a detailed document; major improvements include:

- New analyses of our root biomass data that accounts for uncertainties in ‘background’ root biomass
- Rewriting of the introduction and discussion sections to clarify aims and results of the study
- Inclusion of additional data to further support findings

Thank you for considering our resubmission, we look forward to further discussion and feedback on the revised manuscript.

Sincerely,

Virginia (Gina) Nichols

Assistant Professor
Department of Agroecology
Aarhus University, Denmark

Reviewer #1: Summary: Maize can be grown in a continuous maize system, in a simple rotated maize system, or in an extended maize rotation. Previous research has shown an increase in yield in the extended maize rotation compared to a simple rotated maize system. Differences in resource distribution, microbial communities, and nutrient cycling have also been observed between these two systems. The authors investigate how the extended rotation influences maize root growth and its implications on yield. Over the investigated time period, maize yield is shown to be on average higher in the complex/extended rotation system compared to the simple rotation. This increase in yield also corresponds to an increase in rooting depth, less root biomass reported in the top 15 cm, lower soil penetration resistance, and lower soil moisture in the complex system compared to the simple rotation. The authors claim that the complex rotation system results in a cheaper and steeper root system which helps increase the likelihood the plant can withstand unfavorable growing conditions. They suggest the changes in soil physical, chemical, and biological properties contribute to this resilience.

Major comments:
1. The paper claims that the difference in root growth is due to the increased complexity of the extended rotation. However, it's unclear how the difference in fertilization method (inorganic vs manure) and differences in tillage practices (moldboard plowing after alfalfa and before corn) influence soil physical, chemical, and biological properties. Past studies on the same field acknowledge the possibility that fertilization by manure and moldboard plowing can contribute to differences in soil properties. These factors are mentioned at the end of the discussion, but it would be helpful to mention this in the introduction and in the results when discussing root growth and soil physicochemical properties.

We agree, we reworked the introduction and added text to explicitly acknowledge these complexities.

2. Due to the presence of significant root biomass at maize planting, the authors use the difference in root biomass from the beginning and end of the growing season to compare the two systems. This method leads to a negative root biomass in the 0-15 cm soil depth. While I acknowledge roots from a previous growing season could lead to an overestimate of the maize root biomass, the negative value suggests that a significant amount of the alfalfa roots decomposed by maize maturity. It does not seem accurate to take the difference in this case. Also, given that alfalfa has a favorable C:N ratio and the application of composted manure, the alfalfa roots are in very favorable conditions for rapid decomposition.

This is a good point, and prompted us to rework our root biomass analyses. We instead look at extreme cases of assumed background root decomposition. In all but the most extreme case (no background root decomposition in the short rotation and 100% background decomposition in the extended rotation) the statistics show the short rotation maize produced more root biomass than the extended rotation maize in the 0-15 cm depth. In other words, the conclusion does not change, only the magnitude of the difference. This lends confidence that our results are robust.

3. In addition, the negative root mass in the top 15 cm is ~125 kg ha-1 which accounts for a majority of the difference in total root biomass (~180 kg ha-1) between the two systems. Factoring in that there must be root growth in the top 15 cm that connect the root biomass at 15-30 cm soil depth, I would guess there isn't a significant difference in total root biomass.

Indeed, using our new analytical approach we found there was no conclusive evidence that there was a difference in total root biomass between the two cropping systems, only the way it was distributed in the soil. We added text to clarify this finding in the redrafted manuscript.

4. The root biomass addition method is also confounded by the fact that microbial activity decreases with soil depth. The deeper alfalfa roots may not decompose as readily as those in the top 15 cm. So, subtracting out the root biomass at planting at all soil depths may be accurate at deeper depths but not accurate for shallower soil depths.
By using the 'extreme assumptions' ranges noted above, we addressed this issue. The differences at the deeper soil depths were sensitive to the assumed decomposition, so we do not claim differences between the cropping systems in root production at those depths (>15 cm).

5. The method for collecting root biomass described by the authors likely has significant error and likely contributes the large error bars in Figure 3. Root biomass is collected from 4 samples collected from 32 mm diameter (1.26 inch) soil corers. This is roughly 32 cm2 (5 square inches) area in total. There is likely an extremely high variability in root biomass especially at lower depths where roots are much sparser and would depend on the ability to capture a root in that segment. Thus, it's hard to compare the systems at depths below 15 cm.

We agree with the reviewer that there may have been true differences between cropping systems at depths below 15 cm that we were unable to detect due to our sampling scheme. We added text to the manuscript to acknowledge this possibility. We note that the coefficients of variation of the measurements showed no pattern with regard to depth, so taking more subsamples in each plot may not have reduced the variation at lower depths, and therefore may not have resulted in more statistical power – it's difficult to say.

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7. The differences in root biomass are only reported for 2019 and 2020 when there is not significant difference in yield. It's unclear whether in a year where there is a greater difference in yield results in root biomass trends still hold true.

This is a good point - we added text and additional data demonstrating these patterns in a year with a significant difference in yield to address this concern.

8. In line 162, the authors describe the complex rotation maize roots as "more functional." And, in line 245, the authors claim the complex rotation achieved a more efficient root system with less resource investment. However, roots respond to resource availability as noted by the authors in a separate part of the discussion. Previous publications from the same field experiment note differences in POC, microbial activity, and soil physical properties. It would be more appropriate to frame the difference in root system architecture to be a result of differences in soil properties rather than the maize plant investing in steeper and cheaper roots especially since the maize genotype is the same. In the Lazicki et al., they note that differences in root length density seem to correlate with POC content.

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Minor comments:
1. Degradation of the alfalfa root biomass will impact N and P availability and likely influences rooting. In addition, the weather conditions will influence microbial activity and litter decomposition rates.

Yes, that’s true. To accommodate differences in nutrient addition between cropping systems, N fertilization followed protocols associated with the Late Spring Nitrate Test, and P fertilization followed recommendations based on soil samples drawn from individual plots.

2. In table 3, it's unclear how the timing of maize growth advantage is determined/calculated.

We added a superscript to clarify this – early refers to time periods before the maximum growth rate occurred, and late to time periods after the maximum growth rate occurred.

Also, are the p-value based significances comparing values (ratios and timing) between years? I was confused by how p values were determined for this table.

We added references within the table caption to clarify where the values and significances came from and added text to clarify that the ratios of absolute values are presented only to aid in visual comparisons of the values/patterns.

3. In table 3, please include the values for 2020 and 2019. Even though it is not significant, the root information is mainly for those two years and would help put into context how root growth may impact yield.

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We did not quantify differences in coarse versus fine roots in our study, so we chose not to include this in our discussion.

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Yes, see Figure 2 in Tomer and Liebman (2014, http://dx.doi.org/10.1016/j.agee.2014.01.025).

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8. The higher penetration resistance at depths below 30 cm in the complex system may suggest that the biopores created from deeper alfalfa roots contribute to the differences in rooting depth and the more even distribution of root biomass along the soil profile.

We added this to the discussion. We are unsure whether the biopores would show up in the resistance measurements, so changed the terminology to refer to the penetration resistance of the bulk soil, as we believe that is what is being measured.

Reviewer #2: This study enhances understanding of the 'rotation effect'in agricultural production systems, and provides new data on yields and roots by long term positioning experiment. However, it is unclear what the physiological and ecological mechanisms are, and further supplementary data is needed to support the Conclusions.

Reviewer #3:
This manuscript sets out to show whether changes in root systems explain the rotation effect. It reports many measurements and concludes that chemical and/or biological factors are responsible but physical factors are not. These conclusions at the end of the Discussion are hard to justify because neither chemical nor biological factors were measured.

We rewrote the results and discussion to clarify the evidence that supports our conclusions.

The strength of the manuscript is the extensive data collection, the mostly complete statistical analysis and clear expression. The weakness is that the control of the rooting depth and its connection with yield are not clearly explained. This comes about because the long-term experiment was not designed to clarify the topics discussed in this manuscript. The authors should make revisions that more closely link these data to yield. The catchy title deserves retention; it is a little deceptive because cause and effect are unclear and this should be clarified in the abstract and conclusions.

We hope the redrafting of the discussion section has led to a clearer proposed link between our data and yield. Additionally we adjusted the language in the abstract to clarify our evidence and the conclusions we draw from it.

The manuscript implies that the reported 'complex rotation system' is representative of complex rotation systems in the Midwest US. Clear evidence for such equivalence should be reported but if it is not available the manuscript should address only the particular four-course rotation system that was studied and not extrapolate the results to other, undefined, 'complex' rotations.

This is a valid concern. We believe we address this in the introduction where we define 'general' extended rotations for the Midwestern region of the US as those including a small grain and/or forage:

Midwestern maize-based systems fall into three main categories: (1) continuous maize systems, wherein maize is grown for two or more consecutive seasons; (2) simple rotated maize systems, wherein maize is rotated with soybean; and (3) extended maize rotations, wherein maize is grown in a rotation with two or more years between maize crops, often including a small grain such as oats (Avena sativa) and/or a forage crop such as alfalfa (Medicago sativa).

This applies strongly to references to papers where it is not clear if the complex rotation is identical to the one reported here.

All citations extend from this definition presented in the manuscript:

The maize yield advantage accrued from extending short rotations to include small grains and forage legumes has received less attention compared to the continuous maize penalty, but has likewise been well-documented (Liebman et al., 2008; Stanger and Lauer, 2008; Coulter et al., 2011).

While it is almost impossible for a rotation to be identical due to regional differences in markets, soils, etc. all of our 'extended' rotation references refer to maize rotations that include a small grain and/or forage.

Value-neutral names of the systems would be two-course and four-course rotations.

We changed the terminology to 'short' and 'extended'.

Attributing maize yield to a system is less convincing than to a particular part of the system such as crop species or management operation. It is difficult to unscramble such effects from the data but the authors are in the best position to do so.

We rewrote the introduction and parts of the discussion to make it clear why it is difficult to attribute such a complex characteristic such as yield to a single change, when extending rotations involves a slew of changes that cannot be controlled for individually. We try to present the factors that may be contributing, and in reality is unlikely it is one factor.

The emphasis on nitrogen is unbalanced. It appears on the second line of the abstract and in many parts of the manuscript but there are no reported measurements of soil mineral N. This is a matter of concern since there is much published evidence that crops growing after alfalfa benefit from residual N arising from biological N fixation. If there are no such data available for this experiment, are there published or unpublished data from other studies to fill the gap? The N supply to maize in the four-course rotation also includes a large amount of N in manure, as reported in Table 1 but N-supply is not discussed as a possible reason for the higher yield.

We added citations and text to demonstrate these yield enhancements are observed even when nitrogen is not limiting. We also added text to Table 1 to demonstrate how the amounts of N applied to the two systems were balanced by the side-dressing which was based on a late-spring nitrate test. While the forms of nitrogen supply differed, the absolute amounts did not.

Please provide as much information as possible about the oats and alfalfa; were they cut or grazed and is it possible to report the amount of N contained in alfalfa, both above-ground and below-ground.

We added information indicating the management of the oat straw and alfalfa (oat straw and alfalfa hay were harvested, baled, and removed from the research site, no grazing occurred on these plots). We do not have data on the N content of the below-ground alfalfa biomass. Osterholz et al. 2018 does an in-depth exploration of nitrogen pools in these systems and found they could not explain the differences in maize nitrogen dynamics/yield – we added a more explicit citation of that work.

At L107 there is mention of biological and physical effects on root systems but less prominence is given to chemical factors. The nearest that the manuscript comes to reporting chemical effects is the large amount of manure-N. This should be strengthened.

We added mention of ethylene build up and its potential role in root growth. See above comment concerning N.

The shallow rooting depths (Figs 2 and S2) in some seasons is explained by excess soil water. Could the deeper maize roots in the four-course rotation be due to dewatering of the soil profile by the previous alfalfa crop?

The shallow rooting depth in 2018 was due to high water tables. Our soil moisture sensors at 45 cm depths indicate no difference in soil water contents at that depth between the two rotations, and indicates the soil was fully recharged with water following the alfalfa crop termination the previous fall(s). In general, in this region the entire soil profile goes through a resaturation event before spring's maize planting activities.

The discussion of the effect of alfalfa should include mention of hydrogen fertilisation. Presumably there was hydrogen fertilisation by the alfalfa rhizobia but any contribution by soybean rhizobia would depend on whether they were HUP-plus or HUP-minus.

We considered mentioning this in the original manuscript draft, but in the end felt it was a distraction. The soybean inoculant was HUP-minus, so any differences would be a result of the different quantity of roots releasing H2, and we have no data on the soybean nor alfalfa roots in these plots. While certainly and interesting area of research, we opted to omit it in this manuscript.

The end of the Discussion suggests that roots in 'more complex' rotations are better buffered against unfavorable growing environments. In Fig. 1 the four-year rotation seems to perform best in the wet seasons of 2016-2018. This apparent inconsistency should be discussed.

While the absolute yields of the four-year rotation were highest in 2016 and 2018 as the reviewer points out, we were interested in how the four-year rotation performed RELATIVE to the two-year system. The size of the points on the right panel represents this RELATIVE difference, and it shows that this relative performance was higher in a range of weather conditions (for example in 2013, a hot and dry year, and in 2018 (a hot and wet year).

Detail

Report the statistical significance of the higher yield, rooting depth, root biomass and penetration resistance in the Abstract and Highlights.

Word limits precluded the inclusion of statistical significances in all instances, but we added the significance in several places within the abstract. We hoped that the context would convey that any results reported as being different can be assumed to be significantly so.

The Conclusions (L258) that the result is 'novel' is inconsistent with many papers referred to in the manuscript.

We adjusted that language to better articulate the exact component of our study we feel is novel.

The renumbering of lines makes it difficult to review the manuscript. Please stick to one system in any resubmission.

Yes, we apologize, the line numbers restarted after the landscape table which required a section break. We have corrected this issue.

The references are reported in several different formats and should be standardised.

We corrected the references, switching between citation systems is always a challenge.

Highlights:

- Maize grain yields in the four-year rotation were 8% higher than in the two-year
- Maize roots extended 11% deeper in the four-year compared with the two-year
- Maize had less 0-15 cm root biomass in the four-year compared with the two-year
- Four-year rotation maize roots were ‘deeper and steeper’ compared with the two-year
- Differences were not explained by physical soil measurements

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Matt Liebman reports financial support was provided by Iowa State University Iowa Nutrient Research Center. Matt Liebman reports was provided by Iowa State University Plant Sciences Institute. Sotirios Archontoulis reports was provided by Foundation for Food and Agriculture Research. Sotirios Archontoulis reports financial support was provided by US Department of Agriculture.

The roots of the rotation effect run deep

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Abstract

Context or problem

It is well-established that maize (*Zea mays* L.) grown in extended rotations produces higher grain yields compared to maize grown in one- or two-phase rotations, even when nitrogen (N) is not limiting. Understanding the mechanisms driving this phenomenon, commonly referred to as 'the rotation effect', is important for designing cropping systems that use land and resources efficiently. Differences in root systems can influence resource acquisition and therefore yield, but it is unknown if such differences play a role in the rotation effect.

Research question

We hypothesized that maize grown in an extended rotation system exhibits a deeper root structure with less biomass investment compared to maize grown in a short rotation, and that these characteristics would be correlated with differences in grain production.

Methods

Using a long-term experiment established in 2001, we measured maize rooting depth across the growing season, root biomass in 15 cm increments from 0-60 cm, and grain yields in the maize phase of two contrasting rotations: a 2-year rotation of maize/soybean (*Glycine max* [L.] Merr) using inorganic sources of nitrogen (N) and maximum tillage depths of 15 cm (hereafter the 'short rotation'), and a 4-year rotation of maize/soybean/oat (*Avena sativa* L.)-alfalfa (*Medicago sativa* L.)/alfalfa using a mix of organic and inorganic N sources and inversion tillage to 25 cm (hereafter the 'extended rotation'). Additionally, we measured soil penetration resistance and soil moisture, and performed a growth analysis on aboveground maize biomass.

Results

From 2013-2020, maize grain yields in the extended rotation were equal to or significantly higher than in the short rotation, averaging 8% higher across eight years (11.0 and 10.2 dry Mg ha^{-1}, respectively). In three seasons (2019-2021), the maximum rooting depth of maize in the extended rotation was significantly deeper than in the short rotation by an average of 11% (82 versus 76 cm, respectively), although the timing (e.g., early season, late season) of the extended rotation's maize growth advantage was not consistent across years. At physiological maturity, the two systems had similar amounts of root biomass from 0-60 cm soil depth, but maize grown in the extended rotation invested significantly less of that biomass (30% compared to 47%) into the soil surface layer (0-15 cm). The soil penetration resistances of the two systems differed in a

manner consistent with the differing tillage regimes of the two rotations, however the patterns did not align with root differences.

Conclusions

We posit that the extended rotation's 'deeper and steeper' maize roots did not guarantee higher maize yields, but rather bestowed the plant with more flexibility in resource acquisition which, in certain conditions, resulted in higher grain yields compared to maize grown in the short rotation.

Implications

To our knowledge, this is the first report attempting to mechanistically link rooting patterns with plant growth in the context of the 'rotation effect.' This study enhances our understanding of how cropping system histories impact yields, and provides new data on yields and roots, both of which are highly relevant for sustainable intensification. While the present study focused on physical measurements, it suggests that more detailed exploration of how biological drivers impact root architecture is needed to gain a mechanistic understanding of the 'rotation effect.'

Keywords

Maize; crop rotation, roots, penetration resistance; alfalfa

1 Introduction

Over the past 60 years, the diversity of maize (*Zea mays* L.) production systems in the Midwestern United States (US) has been reduced, moving from multi-species rotations that included small grains and forage legumes to maize monocultures or simple alternations of maize and soybean (*Glycine max* [L.] Merr) (Aguilar et al., 2015; Hijmans et al., 2016; Crossley et al., 2021). Presently, five states in the Midwestern US produce approximately one-sixth of the world's maize and soybean grain (FAO, 2020; USDA National Agricultural Statistics Service 2021), and it follows that in the Midwestern US a significant amount of agricultural land is dedicated to a simplified maize/soybean system (Boryan et al., 2011; USDA National Agricultural Statistics Service Cropland Data Layer, 2021). Several unintended, but nonetheless undesirable outcomes have accompanied this simplification including but not limited to increased rates of soil erosion, increased risk of flooding, and increased risks of nitrate pollution (Hatfield et al., 2009, 2013; Schilling et al., 2010; Jones et al., 2018; Pasley et al., 2021). While cropping system re-diversification may offer avenues for ameliorating many of these issues (Tamburini et al. 2020), there are numerous barriers that currently exist to re-diversifying Midwestern systems (Mortensen and Smith, 2020; Weisberger et al., 2021). Regardless, there is value in understanding the mechanisms that may enable cropping system diversification to contribute to better agricultural outcomes.

Midwestern maize-based systems fall into three main categories: (1) continuous maize systems, wherein maize is grown for two or more consecutive seasons; (2) simple rotated maize systems, wherein maize is rotated with soybean; and (3) extended maize rotations, wherein maize is grown in a rotation with two or more years between maize crops, often including a small grain such as oats *(Avena sativa)* and/or a forage crop such as alfalfa *(Medicago sativa)*. Using these definitions, as maize cropping systems move from monocultures to short rotations or from short to extended rotations, maize yields in the Midwest increase by approximately 10 and 5%, respectively (Crookston et al. 1991; Gentry et al. 2013; Liebman et al., 2008; Davis et al., 2012), and occur even when nitrogen is not limiting (Osterholz et al. 2018; Baum et al. 2023). The former phenomenon is commonly referred to as the ‘continuous maize penalty’, and the latter ‘the rotation effect’. To our knowledge it is unclear whether they are distinct expressions of unique mechanisms, or if they represent a continuum of the same mechanistic underpinnings.

There have been numerous studies in the US Midwest exploring the continuous maize penalty (Dick and Doren, 1985; Peterson et al., 1990; Meese et al., 1991; Crookston et al., 1991; Porter et al., 1997; Varvel, 2000; Stanger and Lauer, 2008; Gentry et al., 2013; Al-Kaisi et al., 2015; Farmaha et al., 2016; Seifert et al., 2017; Vogel and Below, 2018; Bowles et al., 2020). There is evidence it is due, at least in part, to yield suppressive mechanisms in the monoculture system, potentially due to soil biological conditions that may constrain root development and therefore resource capture (Crookston et al., 1988; Johnson et al., 1992; Nickel et al., 1995; Goldstein, 2000). The maize yield advantage accrued from extending short rotations to include small grains and forage legumes has received less attention compared to the continuous maize penalty, but has likewise been well-documented (Liebman et al., 2008; Stanger and Lauer, 2008; Coulter et al., 2011). To our knowledge the mechanisms driving the rotation effect in Midwestern maize systems remain uncertain, partially due to the complex changes in management attendant to extended rotations, rendering it difficult to attribute yield increases to a particular driver.

When above-ground crop products are valued, it is desirable for plants to optimize investments in belowground growth. In nitrogen- or water-limited environments, 'steep, cheap and deep' root ideotypes have been identified as the most efficient use of root investments (Lynch, 2013; Tron et al., 2015; Thorup-Kristensen and Kirkegaard 2016; Thorup-Kristensen et al. 2020). It is therefore feasible that maize grown in extended rotations could also benefit from this root architecture ideotype. Many characteristics of extended rotations may promote deeper crop roots. In a long-term cropping systems research experiment in Iowa (Liebman et al., 2008; Davis et al., 2012) researchers have found differences in the vertical distributions of resources, microbial communities, and nutrient cycling activity in soil profiles of simple and complex maize systems (Lazicki et al., 2016; King and Hofmockel, 2017; Osterholz et al., 2018; Poffenbarger et al., 2020; Baldwin-Kordick et al., 2022), all of which might impact, or be impacted by, root architectures. Indeed, Lazicki et al. (2016) found differences in maize root distributions in plots with varying rotation histories, but the data were limited to shallow depths (0-20 cm) and did not control for previous crop root carryover, which may impact interpretations (Hirte et al. 2017).

We hypothesized that maize grown in extended rotations develops a deeper root structure with less biomass investment compared to maize in a short rotation, and that these differences would be correlated with differences in grain production. To test this hypothesis, we made the following measurements in the maize phase of short (maize/soybean) and extended (maize/soybean/oats-alfalfa/alfalfa) rotations:

1. Maize grain yields from eight growing seasons (2013-2020)
2. Maize root biomass from 0-60 cm soil depths as a proxy for the resources invested by the maize crop into roots in two growing seasons (2019-2020)
3. Maximum maize rooting depth as a proxy for the soil space made available for resource capture by that investment in three growing seasons (2018-2020)

Additionally, we complemented these measurements with detailed aboveground maize growth analysis throughout five seasons (2013, 2014, 2018, 2019, 2020); soil penetration resistance measurements in three seasons (2018-2020); and soil moisture and temperature measurements in two seasons (2018-2019).

2 Methods and Materials

2.1 Study location

The experimental site was located at the Iowa State University Marsden Farm, in Boone County, Iowa, USA (42°01' N; 93°47' W; 333 m above sea level). Dominant soil series were Clarion loam, Nicollet loam, and Webster clay loam, all of which are Mollisols. Before the initiation of the experiment, the site had been managed for at least 20 years with a maize-soybean rotation receiving conventional fertilizer and herbicide inputs. Baseline soil samples (0-20 cm) showed a mean buffer pH of 6.8 and mean organic matter concentration of 51 g kg^{-1} (Liebman et al., 2008). The experiment was arranged as a randomized complete block design with each crop phase of each rotation system present every year in four replicate blocks (Payne, 2015). Plots were 18 m by 84 m, with the entire experiment comprising ~9 hectares. Weather data were collected from a weather station approximately one kilometer from the field site (Iowa Environmental Mesonet, 2021).

2.2 Sampling

This study utilized two of the three maize-based rotations present in the larger study: a 2-year rotation of maize/soybean (hereafter the short rotation), and a 4-year rotation of maize/soybean/oat-alfalfa/alfalfa that periodically received composted cattle manure (hereafter the extended rotation). Oat straw and alfalfa were harvested and removed from the research site; no grazing occurred on the plots. Detailed accounts of plot management are reported elsewhere (Liebman et al., 2008; Hunt et al., 2020) and a summary is presented in **Table 1**. Maize grain yield was measured in 2013-2020, and additional measurements were taken in select years during that period (**Table 2**).

Table 1. Summary of agronomic management of the two rotation treatments housing the maize phase (bolded) sampled in this study; for more details see Liebman et al. 2008 and Hunt et al. 2020

Rotation	**Crop sequence**	**Nitrogen sources**	**Tillage regime**
Short 2-year	Soybean/ **Maize**	Mean total of 180 kg ha^{-1} inorganic nitrogen applied to maize phase, with 112 kg ha^{-1} applied at planting and the remaining at V6 side-dressing based on soil nitrate sampling (Sawyer and Mallarino 2017)	Fall chisel plowing and spring field cultivation preceding soybean planting, spring surface cultivation preceding maize planting
Extended 4-year	Soybean/ Oat-Alfalfa/ Alfalfa/ **Maize**	Mean of 140 kg ha^{-1} organic nitrogen applied as composted cattle manure the fall preceding the maize phase, and 32 kg ha^{-1} inorganic N applied at V6 side-dressing based on soil nitrate sampling (Sawyer and Mallarino 2017)	Fall chisel plowing and spring surface cultivation preceding soybean planting, no tillage events preceding oat/alfalfa planting, fall moldboard plowing of the alfalfa crop followed by spring disking and surface cultivation preceding maize planting

2.3 Statistical analysis

Statistical analyses were conducted using *R version 4.0.2* (R Core Team, 2020) with the *tidyverse* suite of packages (Wickham et al., 2019) or JMP Pro 17.0 (SAS Institute, Cary, North

Carolina, USA). Additional R packages were used for specific analyses as described below. Significance thresholds for statistical tests was set at p=0.05 unless noted otherwise. In all cases several statistical models were fit and compared using Akaike's Information Criteria (AIC; Kuha, 2004) and residual plots, but only the model chosen as providing the best fit is reported. All data used in this manuscript are available in csv format and as an R package available for download (https://github.com/vanichols/maRsden). The R code used to perform these analyses and create manuscript figures is available in a public Github repository (will be made public once manuscript is accepted for publication).

2.4 Grain yields, harvest indices, grain weights, and root-to-shoot ratios

The effect of rotation treatment (short, extended) on maize grain yields was assessed using a linear mixed effects model with rotation, year-factor, and their interaction as fixed effects, and block nested within a year-factor as a random intercept using R package *lme4* (Bates et al., 2015). The R packages *emmeans* (Lenth et al., 2018) and *lmerTest* (Kuznetsova et al., 2017) were used for comparisons and statistical summaries of the model, using a Tukey adjustment for multiple comparisons.

Differences in harvest indices, 500-grain weights, and the root-to-shoot ratios were assessed using a linear mixed effects model with rotation, a year-factor, and their interaction as fixed effects and a random block intercept, with a Tukey adjustment for multiple comparisons.

2.5 Rooting depth

We modeled rooting depth as a function of the cumulative maize growing-degree-days (GDDs) accrued since planting (base temperature 10° C, maximum temperature 30° C) to facilitate comparisons between years. Non-linear models were fit using the R package *nlraa* package (Miguez, 2021). We tried several non-linear models fit to both the raw data and the data filtered to remove measurements taken after the season's maximum depth had been reached (**Figure S1**). We found a three-parameter logistic curve (Archontoulis et al., 2015) fit to the filtered data produced the best fit according to AIC and inspection of residuals:

Eqn. 1 $$rootdepth(GDD) = \frac{A_{sym}}{(1+\exp\left(\frac{(x_{mid}-GDD)}{scal}\right))}$$

where *rootdepth(GDD)* is the maximum rooting depth at a given cumulative GDD after planting and A_{sym}, x_{mid}, and *scal* are estimated parameters. A_{sym} represents the maximum rooting depth achieved, x_{mid} represents the cumulative GDD value at which half of the maximum rooting depth was achieved, and *scal* describes the steepness of the curve (Miguez et al., 2018). We found the best model fit incorporated a fixed effect of rotation on A_{sym}, $scal$, and x_{mid}; a random effect of a year-factor on A_{sym}, x_{mid}, and *scal*; a random effect of block on A_{sym}; and a power variance structure (Pinheiro and Bates 2022).

Table 2. Summary of measurements used for this study

Measurement	Years	Description
Maize grain yield	2013-2020	Maize grain yield was determined using a 6-row combine equipped with a yield monitor and moisture meter. Sampling areas for yield were approximately 4.6 m x 84 m and were six rows away from plot edges. All yields are reported on a dry weight basis.
Maize above-ground biomass	2013, 2014, 2018, 2019, 2020	Maize above-ground biomass was measured approximately every two weeks throughout the season. Eight plants were cut at ground level, separated into leaf, stem, and reproductive components, dried at 60° C for at least 72 hours, then weighed.
Maize maximum rooting depth	2018, 2019, 2020	Maximum maize rooting depth was measured starting at 20, 23, and 41 days after planting in 2018, 2019, and 2020, respectively. Sampling was repeated approximately every 2 weeks until 84, 106, and 151 days after planting in 2018, 2019, and 2020, respectively. Maximum rooting depth was determined visually using the protocol of Ordóñez et al. (2018). A soil core was drawn with a 19-mm-diameter soil probe, white roots were identified, and their depth recorded to the nearest cm using a meter stick. At a given date, samples were taken repeatedly from the same core location until no white roots were identified in the core. Four cores were taken per plot at each sampling date.
Soil moisture and temperature at 15 and 45 cm depths	2018, 2019	5TM soil moisture and temperature sensors (METER Group Inc., Pullman, WA, USA) were installed at two depths (15 and 45 cm) in each plot. Data were recorded hourly during the growing season using EM50 data loggers.
Penetration resistance	2018, 2019	Penetration resistance was measured using a FieldScout 900 Soil Compaction Meter (Spectrum Technologies, Inc., Aurora, IL, USA). Values were recorded every 2.54 cm to 45 cm depth immediately following planting and/or approximately 60 days after planting. Ten measurements were taken randomly throughout each plot. Measurements were taken within two days after a saturating rain to avoid capturing differences due to soil moisture.
Maize root mass 0-60 cm	2019, 2020	Maize root mass to a soil depth of 60 cm was measured at 3 and 105 days after planting in 2019, and at 4 and 117 days after planting in 2020. Four soil samples per plot were drawn at a location 10 cm from maize rows in 15 cm depth increments using a 32-mm-diameter soil probe. Soil from each depth increment within a plot was composited and roots were recovered using a sequence of elutriation (washing), flotation, recovery from organic debris using tweezers and a stereo microscope, drying, and weighing (Dietzel et al., 2017).

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2.6 Root mass

Distinguishing newly added root biomass from the previous year's crop and weed root biomass is difficult using visual sorting, and failure to address these background levels of roots can lead to overestimates of new root mass, with the overestimate varying by cropping history (Hirte et al. 2017). To address this issue, the three main options currently available to field researchers include the use of isotopes, correction for background levels of root material by using a baseline measurement or root growth cores, or maintaining a crop- and weed-free area to track background levels throughout the season. We chose to take a background sample shortly after the maize crop was planted. Additional details of sampling protocols are presented in **Table 2**. When interpreting the data, we used the two extreme assumption cases concerning the percentage of the sampled background roots that decayed between the start of the maize growing season (**Table 2**) and the last sampling date near maize physiological maturity: 0% background root decay, and 100% background root decay. The true amount of background roots present at the time of sampling falls between the extreme assumptions, and we therefore report the production of new crop root biomass as a range of possible values.

The Restricted Maximum Likelihood (REML) method for linear mixed effect models in JMP Pro 17.0 was used to evaluate the effects of rotation treatment on maize root mass production within four soil depth increments (0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm) for each of the extreme assumption cases. Year, rotation system, soil depth, and date were treated as fixed factors, and block and its interactions with the fixed factors were treated as random effects. Sampling depth was nested within year and rotation. Due to the inherently high variability of root measurements, for these analyses the threshold for investigating contrasts (as allowed by degrees of freedom) was set at $p<0.10$.

2.7 Growth analysis

We modeled above-ground biomass as a function of day-of-year using a three-parameter logistic curve (Eqn. 1), as it provided the best fit of all the non-linear models assessed (logistic, Richards, Gompertz, Weibull, Beta; Miguez et al. 2018) based on AIC criteria. A model using GDDs produced similar results, but we chose to report the model using calendar days because it was conceptually easier to compare systems. A separate model was fit for each rotation in each year to allow for derivatives to be taken for each rotation in each year. For visualization purposes, the first- and second-order derivatives of the fitted equation were used to visualize the absolute and relative growth rates over time, allowing for visualization of when differences in growth rates occurred rather than relying on parameter interpretations alone. The effect of rotation on fitted parameters was assessed using the *confint* function of base R.

2.8 Root-to-shoot ratios

A root-to-shoot ratio for each experimental unit was estimated using the range in possible root additions in the 0-60 cm soil profile and the maximum above-ground biomass as predicted by the growth analysis (see Section 2.7). In Iowa, maize roots in the 0-60 cm depth increment account for more than 90% of total root mass (Nichols et al. 2019) but will nonetheless provide an underestimate of the root-to-shoot ratio (Ordóñez et al. 2020). We therefore calculated the ratio to be interpreted as relative, rather than absolute, values.

2.9 Penetration resistance

Penetration resistance was statistically modelled separately for each year and date of sampling using a generalized additive mixed model with a fixed intercept effect of rotation treatment, a fixed 'wiggle' component of rotation treatment, five knots, and a random 'wiggle' effect of block. Generalized additive models can model highly non-linear relationships and are useful when the goal is to compare treatments rather than to create predictions. The *gamm* function of the R package *mgcv* (McCulloch and Neuhaus, 2005; Wood, 2011) was used, and the *emmeans* package was used to assess pairwise comparison significance. Models were fit using both the raw and square-root-transformed data. Although the model on the transformed data produced a better fit according to inspection of residuals, statistical conclusions were not different in the two models so the results from the untransformed data are presented for ease in interpretation.

2.10 Soil moisture and temperature

The hourly soil moisture and temperature data were averaged over 24-hour periods (12:00 am to 11:59 pm) for analysis. The daily means were modelled statistically as a function of day-of-year separately for each year and depth using a generalized additive mixed model with a fixed intercept effect of rotation treatment, a fixed 'wiggle' component of rotation treatment, 35 knots, and a random 'wiggle' effect of block using the R package *mgcv*.

3 Results

3.1 Grain yields and weather

Maize yields ranged from 7.0 to 12.7 dry Mg ha^{-1} over the 2013-2020 study period. The effect of rotation depended on year ($p<0.001$; **Figure 1**), with the maize grown in the extended rotation producing significantly higher ($p<0.01$) grain yields in five (2013, 2014, 2016, 2017, and 2018) of the eight years. Averaged over all years, maize grown in the short rotation yielded a mean of 10.2 dry Mg ha^{-1}, while maize grown in the extended rotation yielded 8% higher with a mean grain yield of 11.0 dry Mg ha^{-1}.

Over the past 30 years, from 15-April through 15-October the research site averaged 597 mm of precipitation with a mean air temperature of 19° C. From 2013-2020, growing season weather conditions varied considerably compared to these long-term averages, with the grain yield dataset capturing conditions in all four temperature/precipitation combinations (**Figure 1**). Years that included growth analyses were likewise represented in all four quadrants, and the three years with full datasets (grain yields, growth analysis, root data) represented all but cool and wet conditions.

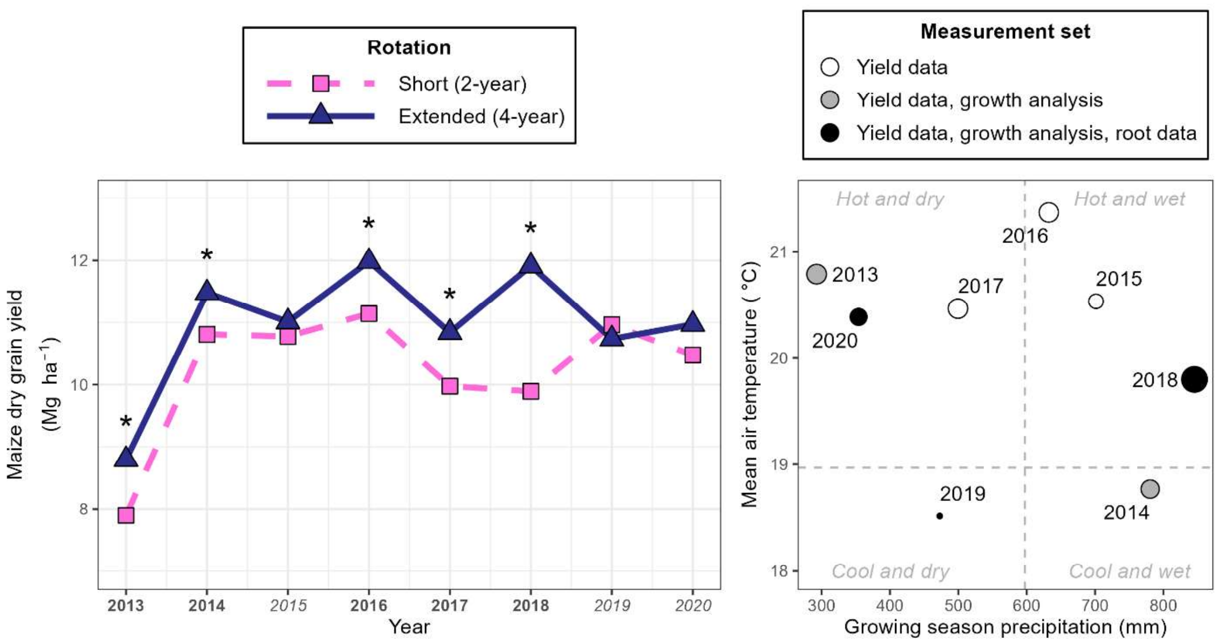

Figure 1. Maize yields and growing season weather; (left) mean maize grain yields (n = 4) in the extended (dark blue, solid, triangles) and short (pink, dashed, squares) rotations from 2013-2020 (lines connecting points for ease in viewing), years with significantly different yields (p<0.01) indicated with asterisks and bolded year font; (right) growing season precipitation and mean air temperatures of each measurement year as compared to 30-year means (dotted lines) with size of points proportional to the extended rotation's maize mean yield advantage over the short rotation, and point color representing the measurement set for that year.

3.2 Rooting depth

The rooting depth of the maize grown in the extended rotation trended consistently deeper in the majority of sampling times in all three growing seasons (**Figure 2**). The maximum rooting depths in 2018 were shallow (~50 cm) due to an extremely wet year (**Figure 1**) that caused consistently shallow water tables as documented at a nearby experimental site (Ebrahimi et al. 2019). Rotation affected maize maximum rooting depth (A_{sym}; $p<0.01$; **Table S1**), estimated at 11% deeper in the extended rotation compared to the short (82 cm and 76 cm, respectively). While the extended rotation roots also descended faster, the effect was not statistically significant (x_{mid}; $p=0.19$).

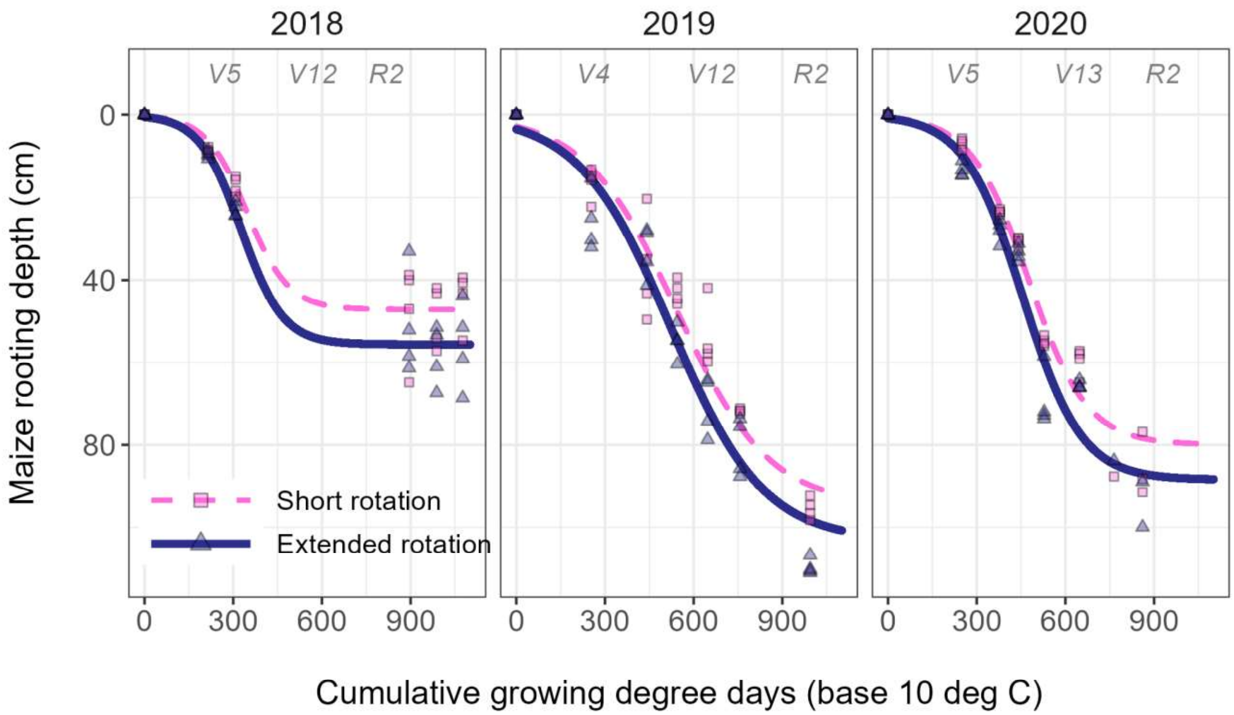

*Figure 2. Maize rooting depth across three growing seasons (2018, 2019, 2020; refer to Figure 1 for weather conditions) in short (pink, dashed, squares) and extended (dark blue, solid, triangles) rotations; points represent individual measurements, lines represent the non-linear model fits, italic text is the maize stage; model uncertainty is omitted for visual simplicity but can be viewed in supplemental material (**Figure S1**); first points at 0 depth indicate maize planting date.*

3.3 Root mass

Root mass present at maize planting (hereafter 'background' root mass) ranged from 68 to 2753 kg ha^{-1}, with the extended rotation tending to have higher amounts (**Table S2**). Year interactions were not significant, (**Table S2**), so results are presented as marginal estimates over years. At the 0-15 cm depth increment, the root mass added in the two systems differed significantly ($p = 0.02$; **Table S3**), regardless of background root decomposition assumptions; the short rotation added between 314 (standard error of the mean [SE]:110) and 506 (SE:56) kg ha^{-1}, while maize grown in the extended rotation added between 101 (SE:110) and 321 (SE:55) kg ha^{-1} of root mass (**Figure 3**). The extended rotation's lower maize biomass relative to the short rotation in the top 15 cm was counterbalanced by higher biomass relative to the short rotation in soil layers deeper than 15 cm (**Figure 3**). Although these differences were not significant (**Table S3**), this pattern resulted in the total root biomass of the two rotations being statistically equivalent. Dietzel et al. (2017) studied maize root growth using in-growth cores and found maize added 480-560 kg ha^{-1} in root material over the growing season in the top 30 cm of soil, suggesting the ranges found in our study are reasonable (**Figure 3**).

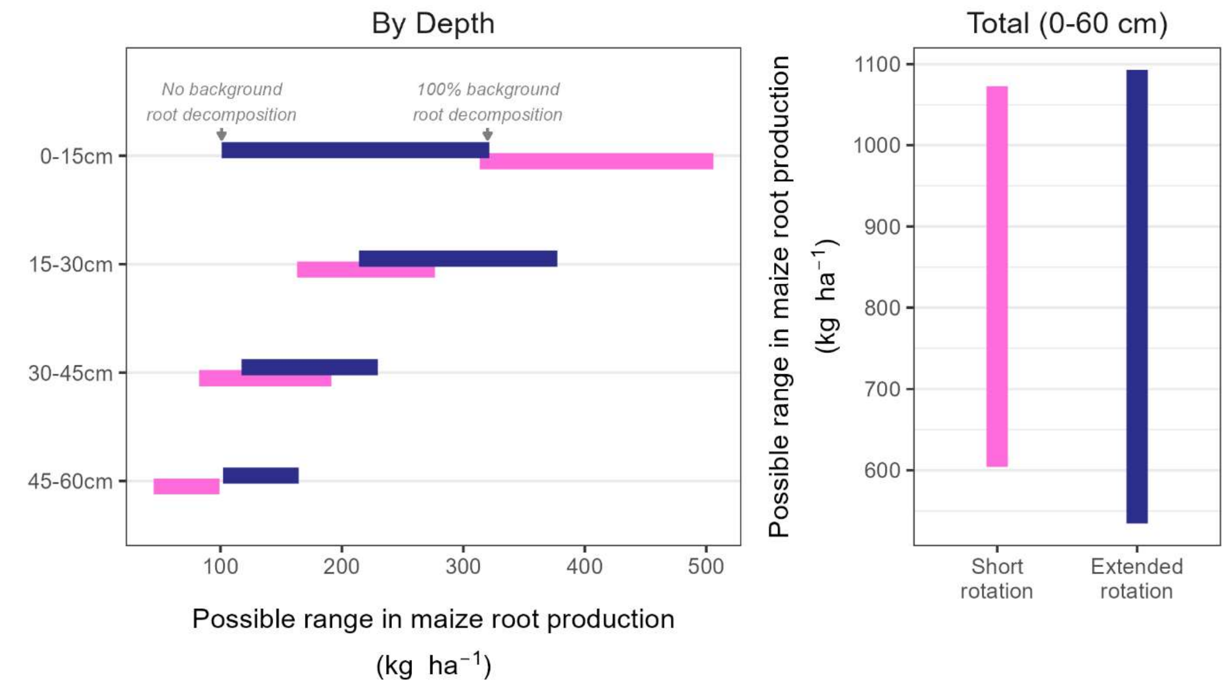

Figure 3. (Left) Possible ranges in mean maize root mass production (n=8) depending on assumed rate of decomposition of root mass present at planting ('background' roots) for maize grown in the short (pink) and extended (dark blue) rotations separated by depth and (right) range in total possible root biomass for 0-60 cm. Data are averages from two growing seasons.

3.4 Growth analysis

The maximum aboveground maize biomass (*Asym*; Eqn. 1) as estimated from the growth analysis was significantly higher in the extended rotation in the years when the extended rotation yielded significantly higher grain yields, but were not different in years without significant differences in grain yields (**Table 3; Figure S2; Table S1**). The date at which the maize achieved half of its maximum biomass (*xmid;* Eqn. 1) was significantly earlier in the extended rotation in 2013 ($p = 0.05$) and exhibited higher absolute growth rates compared to the short rotation before maximum growth rates were achieved (e.g. early in the season; **Figure S2**). Conversely, *xmid* for the extended rotation occurred significantly later than for the short rotation in 2018 ($p < 0.01$) and had higher absolute growth rates after maximum growth rates were achieved (e.g. later in the season). The timings of growth were not significantly different in the other three years with growth data. The maximum growth rates in the two rotations (*scal;* Eqn. 1) were not significantly different in any of the five years. The harvest index and 500-kernel weights were consistently higher in years with large rotation effects on grain yield but did not differ in years that lacked a strong rotation effect (**Table 3**).

The root-to-shoot ratios ranged from 0.002 to 0.015 depending on the assumed background root decomposition (**Figure S3**). Trends were inconclusive due to the variation produced by the assumptions leading to calculation of the ratios. While these root-to-shoot ratios are lower than those reported in literature for maize (e.g., 0.09 from Ordóñez et al. 2020), our root measurement methodology and driving questions differed, and our total root biomasses are consistent with other studies that control for background root amounts (e.g. Dietzel et al. 2017).

Table 3. Summary of characteristics of maize in the extended rotation relative to maize in the short rotation, including grain yields (Figure 1), root characteristics (Figures 2, 3) growth analysis (Figure S2, Table S4), and yield components (Figure S2) for years with growth analysis data, ordered by magnitude of grain yield differentials; ratios of values are presented to simplify visual comparisons of rotations

Year	**Ratio of extended:short**	**Extended rotation's maize maximum**	**Timing of extended rotation's**	**Ratio of extended:short**	**Ratio of extended:short**

	rotation maize grain yield†	rooting depth/surface roots relative to short rotation	maize growth advantage‡	rotation harvest index	rotation 500-kernal weight
2018	1.20	Deeper/-	Late season	1.08	1.13
2013	1.10	-	Early season	1.04	-
2014	1.06	-	ns	ns	-
2020	ns	Deeper/steeper	ns	ns	ns
2019	ns	Deeper/steeper	ns	ns	ns

†Ordered by largest to smallest estimated rotation effect on maize grain yield
‡Early season refers to periods before the maximum maize growth rate occurred, late season to periods after
* ns: not significant at p=0.05
- indicates those data were not collected that year

3.5 Penetration resistance

Penetration resistance above 30 cm soil depth was consistently lower in the extended rotation compared with the short rotation, regardless of year or sampling period (planting, late season; **Figure S4**). From 0-30 cm, the extended and short rotations had mean penetration resistances of 0.6 and 0.7 MPa at planting, and 1.1 and 1.5 MPa at late season sampling, respectively, corresponding to a 20% lower penetration resistance in the top 30 cm. From 30 to 45 cm, on average the extended rotation had higher penetration resistance compared to the short by an average of 22% (1.1 MPa/0.9 MPa at planting, and 1.7/1.4 MPa in the late season, respectively).

3.6 Soil moisture and temperature

In both years of measurement, soil moisture at 15 cm depth in the extended rotation was significantly lower than in the short rotation for the first month following planting (**Figure S5**). After that period, the effects of rotation on soil moisture were not consistent across years; in 2018 the short rotation soil was consistently wetter than the extended rotation at both 15 cm and 45 cm depths, but in 2019 there was no difference. In 2018, the extended rotation's lower soil moisture at 15 cm was concomitant with a significantly higher soil temperature compared to the short rotation by ~0.5 deg Celsius, but otherwise showed no consistent trends (**FigureS5**).

4 Discussion

Compared to maize grown in a short rotation, we found maize grown in an extended rotation had consistently deeper maximum rooting depths during three years of measurement in widely varying weather conditions (**Figures 1 and 2**), and a root system that was more evenly distributed from 0-60 cm in two years of measurements (**Figure 3**), corroborating evidence from Lazicki et al. (2016) (**Figure S6**).

The hypothesis that these 'steeper and deeper' root characteristics are a consistent feature of the extended rotation requires further testing, but the data collected in the present study provide support for such a hypothesis. These potentially consistent differences in root characteristics did not, however, significantly enhance grain yields in every year (**Figure 1**). We posit that through changes in physical, chemical, and biological soil properties, the extended rotation provides opportunities for maize roots to grow 'deeper and steeper,' possibly positioning the plant to access resources (i.e., water and nutrients) that may be, or may become, available in deeper soil layers. The feasibility of these interactions is supported by a simulation model that varied root front velocities in maize (and therefore maximum rooting depths); the simulation showed that the impact of deeper rooting on maize yields depended heavily on the year, and that the magnitude of yield impact was comparable to that which we observed (unpublished data; **Figure S7**).

The reasons for the 'steeper and deeper' root architecture are not clear. In a previous study, in which Bay et al. (2021) drew soil from the top 20 cm of plots in each rotation system, sieved and homogenized the soil, packed it into rhizotrons at the same bulk density, and grew maize for 21 days, the investigators found that even in the homogenized soil, maize grown in soil from the extended rotation had a maximum rooting depth ~30% deeper compared to the maize grown in the short rotation soil, as well as significantly thinner but more numerous roots. Working with the same plots as in our study, King and Hofmockel (2017) found microbial biomass was both higher and more evenly distributed in the top 20 cm of the extended rotation soil compared to the short rotation, which, if biological drivers are realized in the field, could be contributing to the differences in root distributions seen in the present study. Results from Bay et al. (2021) and King and Hofmockel (2017), combined with the lack of correlation between root distribution patterns and physical measurements (soil penetration resistance, soil temperature, soil moisture) seen in our field study, provide support for the hypothesis that biological and/or chemical drivers in the soil contributed to root differences between maize in the extended- and short rotations.

Nonetheless, while biological and chemical drivers likely play a role, physical drivers may still be contributing to the 'steeper and deeper' maize roots in the extended rotation in the field. The root legacy of the crop preceding maize (alfalfa in the extended rotation, soybean in the short rotation) might have affected the maize root distributions. One study using mini-rhizotrons observed that when a maize crop followed alfalfa, the maize root distribution closely mimicked the alfalfa root distribution, with 41% of the maize roots following old alfalfa root channels (Rasse et al. 1998). Alfalfa root systems tend to be deeper than those of annual crops (Fan et al. 2016), and even with moldboard plowing (20-25 cm depth) used in our study, there would be intact decaying alfalfa root channels that the maize roots may have followed.

The extended rotation had higher penetration resistances from 30-45 cm depths compared to the short rotation, indicating differences in penetration resistance in bulk soil cannot explain the deeper rooting depths in the extended rotation. Previous work has shown that depth to the water table is associated with differences in maize roots (Nichols et al. 2019), and measured water table heights at this site in 2019 (unpublished data) showed the rotation treatment had no discernible effect on water table dynamics, indicating the differences in root distributions between the treatments were not due to differences in the depth to the water table.

Previous studies have found lower bulk densities and higher particulate organic matter in the extended rotation, which likely reflect the moldboard plowing of the alfalfa crop during the fall prior to maize planting as well as greater particulate organic matter from manure additions (Lazicki et al. 2016; Poffenbarger et al. 2020; Baldwin-Kordick et al., 2022). This is consistent with the lower penetration resistances observed in the present study in the top 30 cm, and likely explains the lower soil moisture observed in the extended rotation at planting. While neither system had absolute resistances high enough to meaningfully impede root penetration, the observed differences in penetration resistance in the surface layers (0.1-0.4 MPa) were of a magnitude that could potentially affect root elongation; a study done with intact soil cores found resistances of only 0.3-0.5 MPa reduced maize seeding root elongation by 50-60% in a sandy loam soil (Bengough and Mullins, 1991).

While the rotation benefit observed in the present study was realized in a range of weather conditions (**Figure 1**), it was large in both the wettest (2018) and driest (2013) years. Many climate change scenarios project increased occurrences of precipitation extremes (Zhang et al. 2013), suggesting extended rotations may play an important role in building cropping system resilience to climate change, and that resilience may be due in part to differences in root architecture.

5 Conclusion

This study provides novel evidence that growing maize in extended rotations can result in changes to maize root structure including maximum rooting depths and vertical distributions. The observed changes in root structure may have affected below-ground resource acquisition by the crop, which may in turn have resulted in higher maize grain yields under certain conditions. Differences in penetration resistance and previous crop root legacies may contribute to altered maize root patterns, but there are likely additional biological and/or chemical drivers that are not well understood in field settings, and would benefit from targeted research. Potential impacts of this research include fine-tuning of crop models to account for cropping history-induced differences in maize rooting depth, investigating if/how genetics interact with extended rotations to further augment the benefits of deeper/steeper rooting in the Midwest, and investigating whether the patterns observed in this study hold in other crops.

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8 Author contributions

All authors contributed to data collection, GN and ML performed data analyses, GN wrote the initial draft of the manuscript, all authors contributed to editing of the final manuscript.

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10 Appendix. Supplementary Material

The following figures are included as supplementary material:

Figure S1 – Maximum rooting depth over time, raw dataset and unsmoothed fitted values

Figure S2 - Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight

Figure S3 – Root to shoot ratios for 2019 and 2020

Figure S4 - Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference

Figure S5 - Soil moisture and temperature at 30 and 45 cm depths in the maize phase of the short and extended rotations

Figure S6 – Data from Lazicki et al. 2016

Figure S7 – Simulated impact of changes in root front velocity on maize yield

The following tables are included as supplementary material:

Table S1 – Summary of above-ground growth-analysis and rooting depth non-linear parameter fits

Table S2 - Summary of 'background' root samples taken shortly after maize planting

Table S3 - Summary of fixed effect tests on root mass

Table S4 - Summary of contrasts for the complex versus short rotation for root mass added at each depth

704

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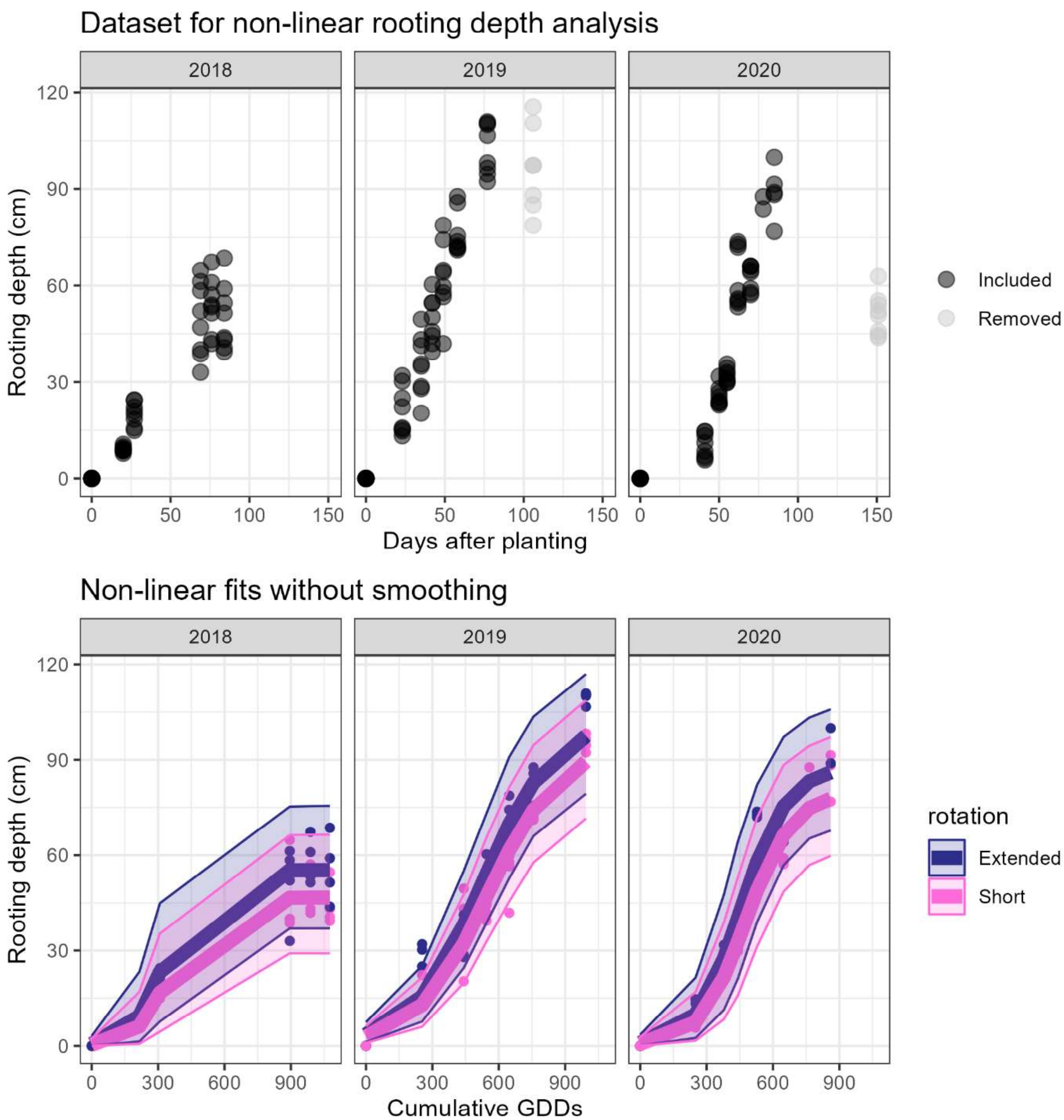

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707 ***Figure S1** – Maximum rooting depth over time, raw dataset and unsmoothed fitted values*

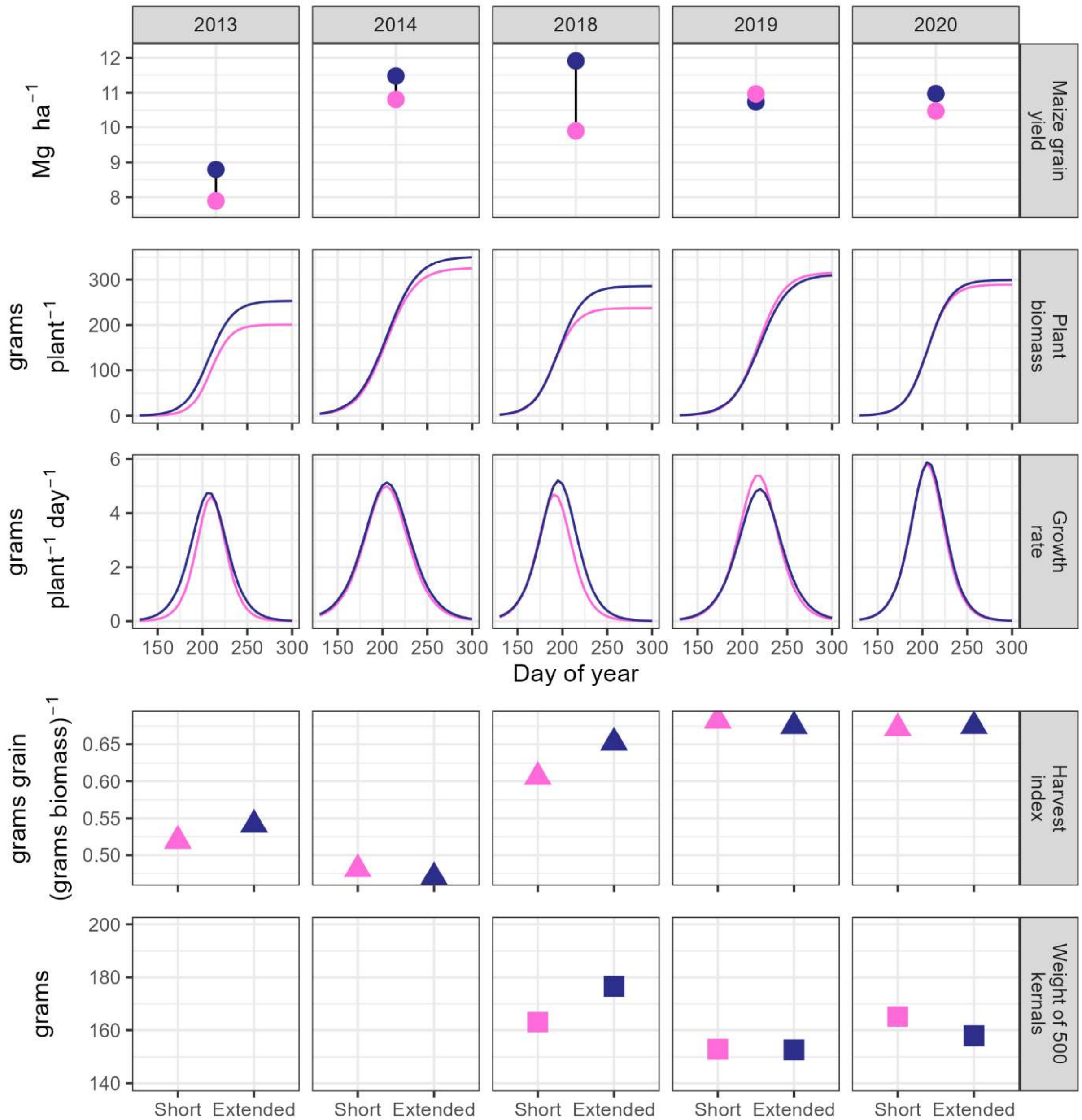


***Figure S2**. Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight*

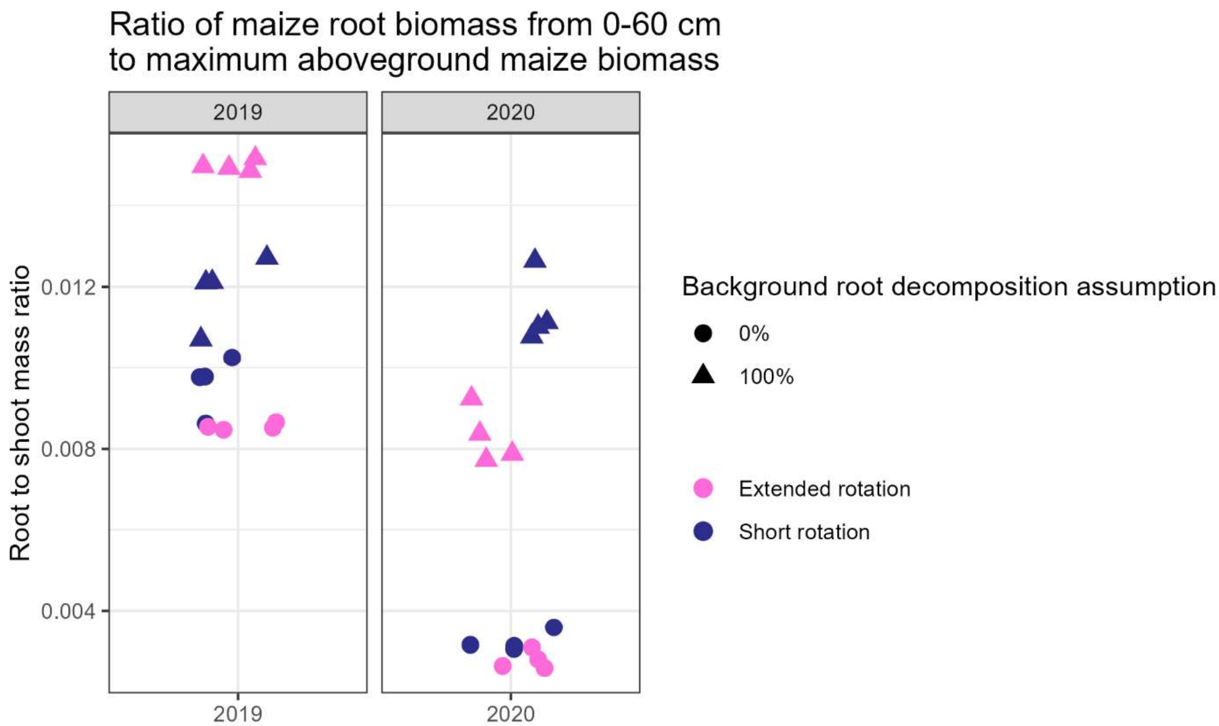


Figure S3. Root to shoot ratios for 2019 and 2020, with different background root decomposition assumptions

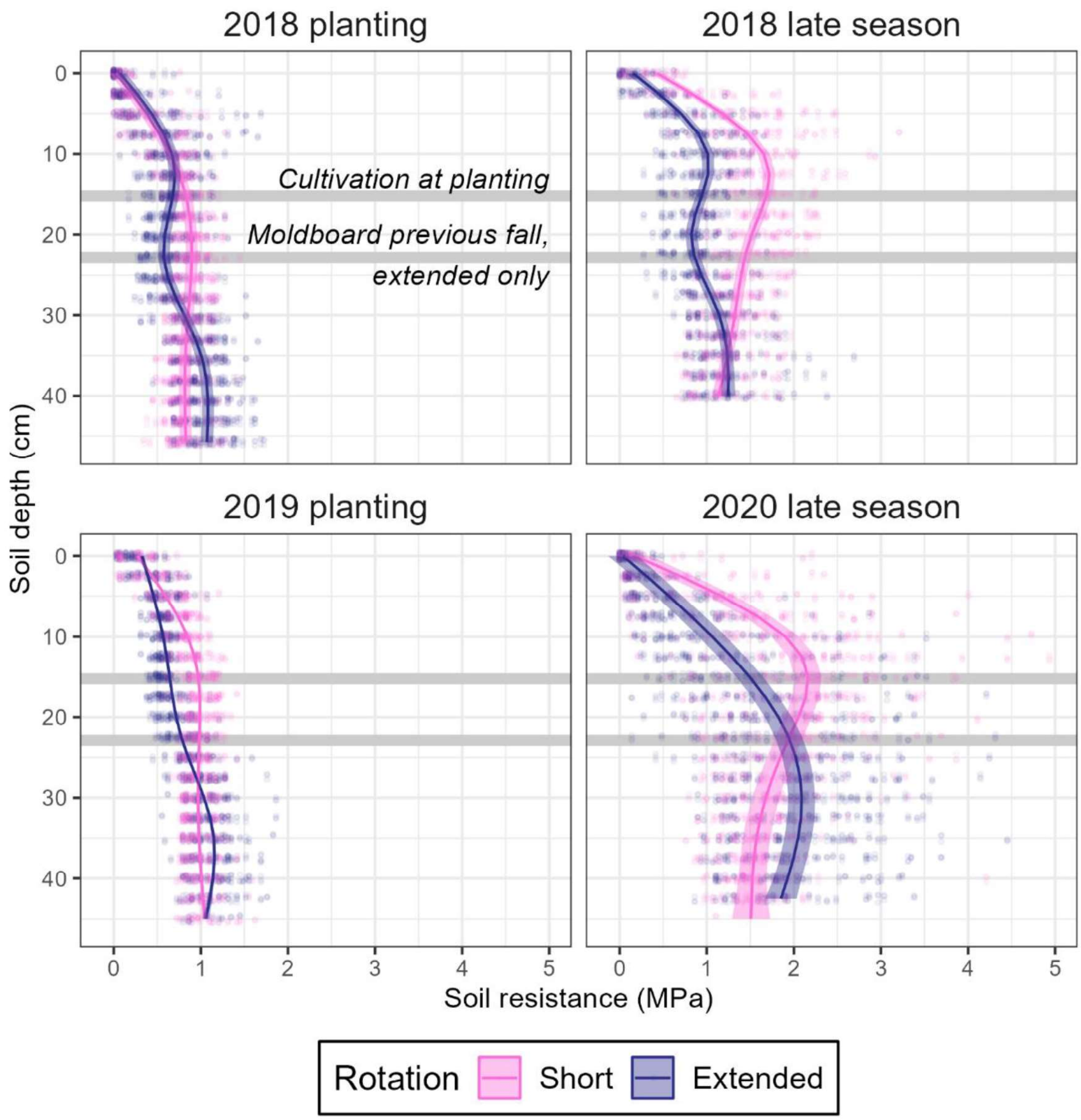
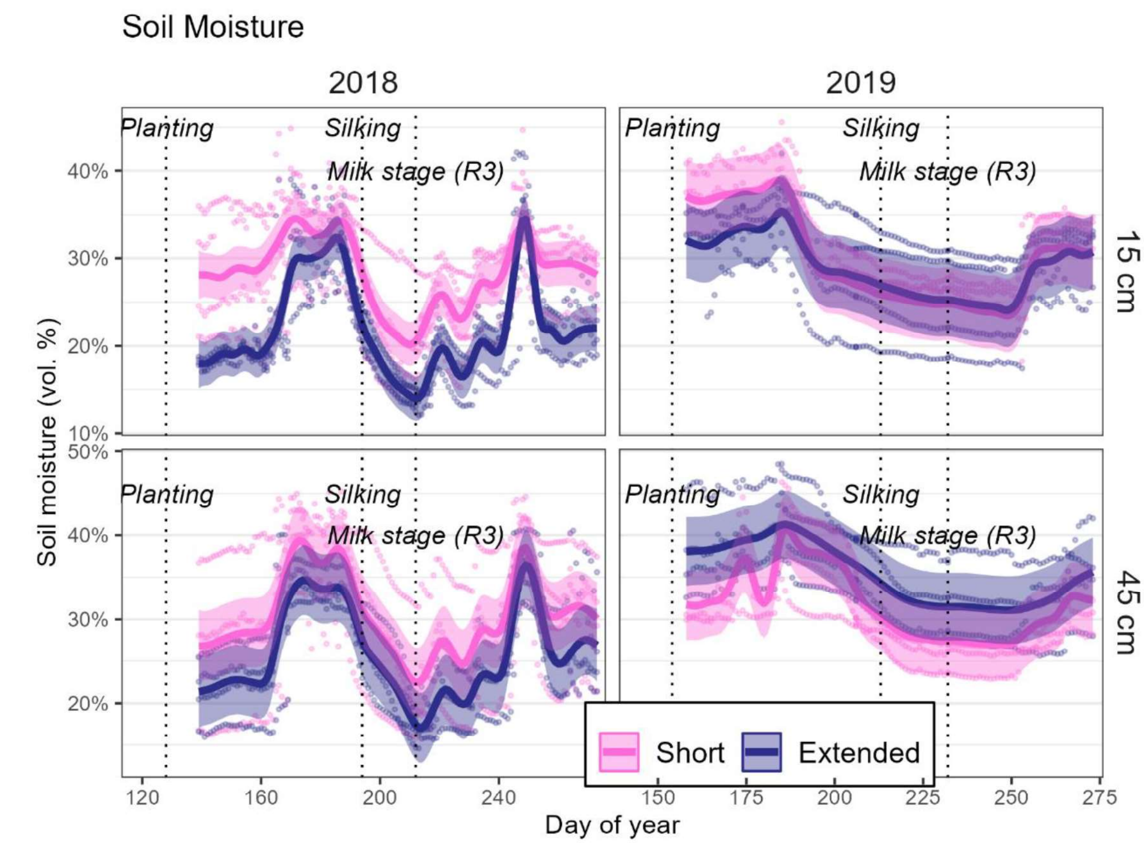
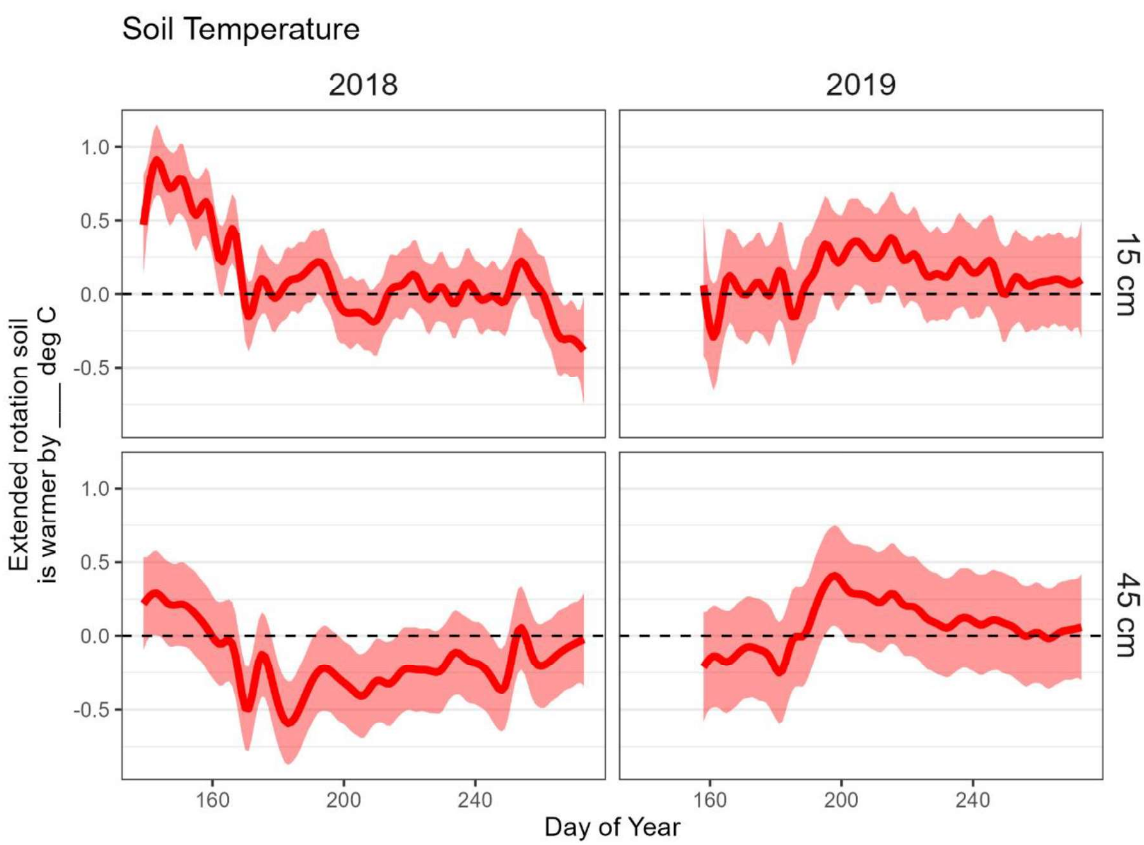


Figure S4. Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference


Soil Moisture
2018
2019
Planting
Silking
Milk stage (R3)
Soil moisture (vol. %)
10%
20%
30%
40%
50%
15 cm
45 cm
Short
Extended
120
160
200
240
150
175
200
225
250
275
Day of year


Soil Temperature
2018
2019
Extended rotation soil
is warmer by ___ deg C
1.0
0.5
0.0
-0.5
15 cm
45 cm
160
200
240
Day of Year

721

Figure S5. Soil measurements at 30 and 45 cm depths in the maize phase of the two rotations; (top) soil moisture in the extended (dark blue) and short (pink) rotations, points represent individual sensor values, lines the estimated values, and ribbons the 95% confidence interval around the estimates; (bottom) differences in soil temperature in the extended rotation compared to the short rotation as estimated by GAM with 95% confidence intervals

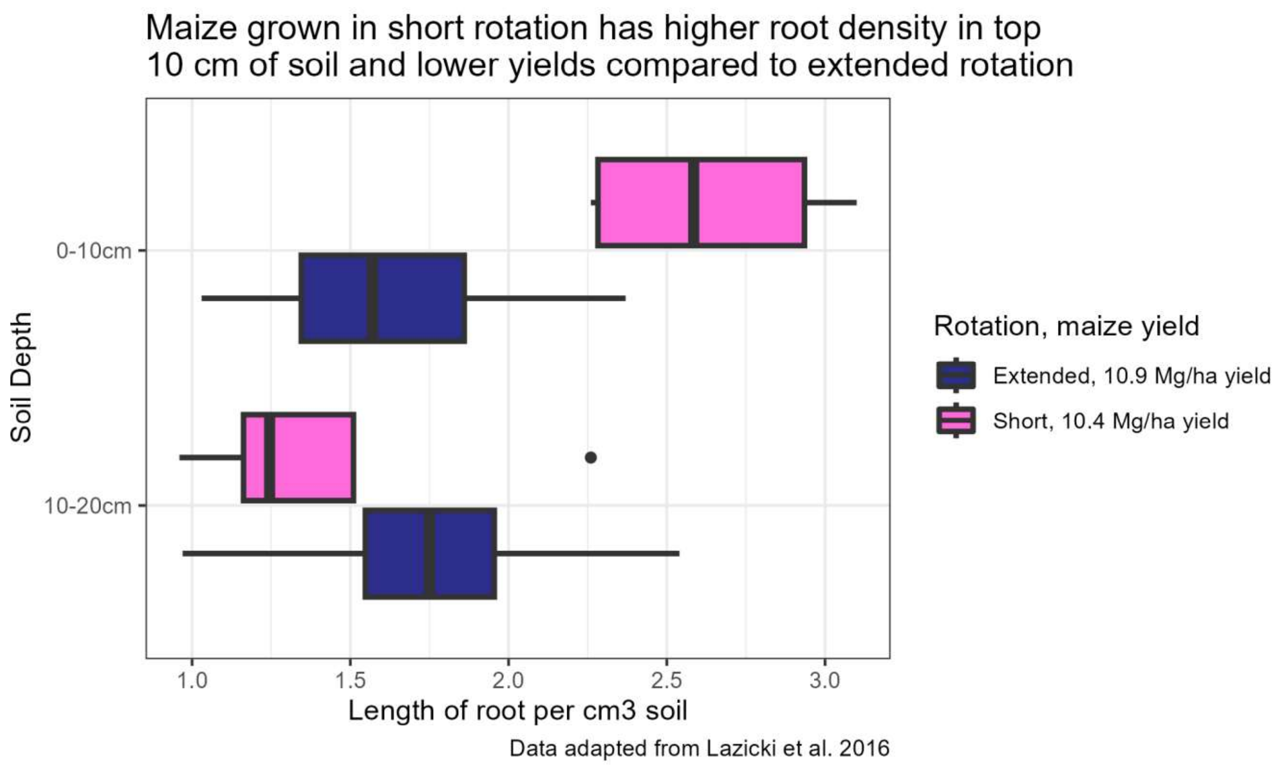


Figure S6 – *Data from Lazicki et al. 2016 shows differences in maize root structures coinciding with maize yield differences in short and extended maize rotations in 2009*

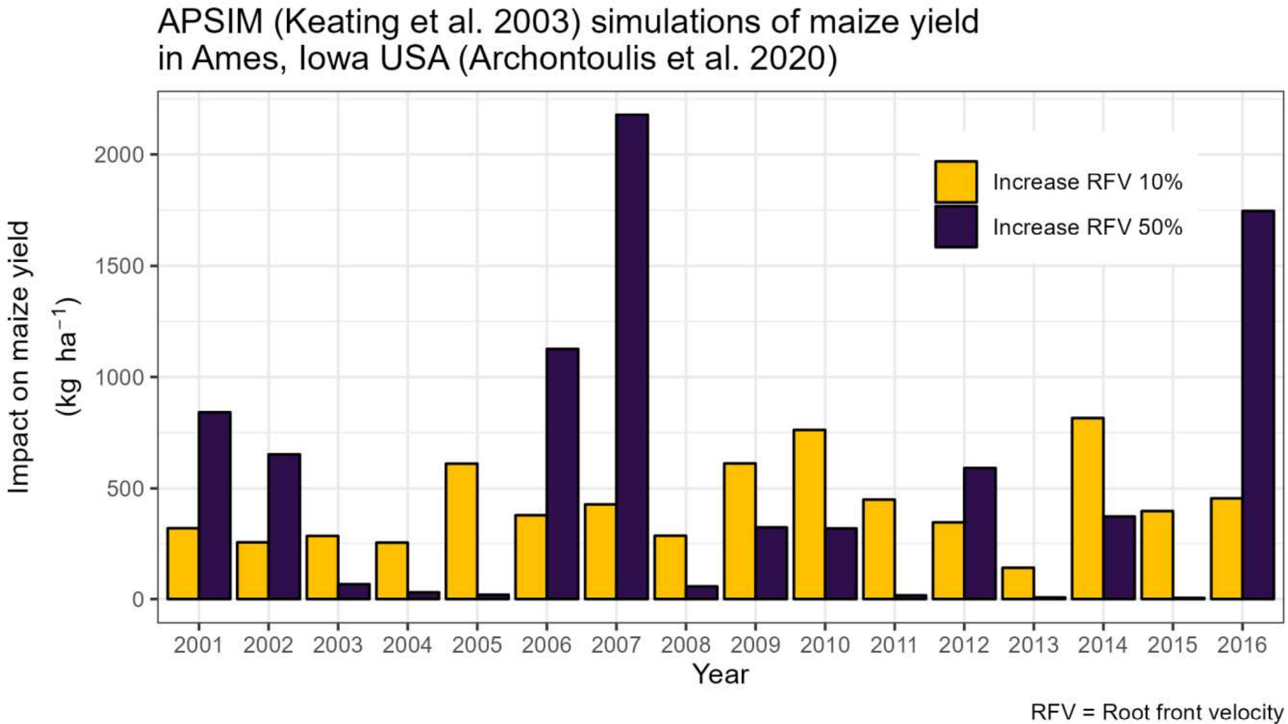


Figure S7 *– Simulated impact of changes in root front velocity on maize yield. Simulations are derived from results supporting the publication of Archontoulis et al. (2020), and the reader is directed to that publication for more details*

Archontoulis SV, Castellano MJ, Licht MA, Nichols V, Baum M, Huber I, Martinez- Feria R, Puntel L, Ordóñez RA, Iqbal J, Wright EE. Predicting crop yields and soil- plant nitrogen dynamics in the US Corn Belt. Crop Science. 2020 Mar;60(2):721-38.

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Table S1. *Summary of 3-parameter logistic curve fits for rooting depth analyses and above-ground growth-analyses, parameters that differed significantly by rotation ($p<0.05$) are bolded.*

Year	Rotation	Asym	xmid	scal
		(cm)	*(GDDs)*	
Rooting depth analysis				
	Short	**74 (CI:50-98)**	455 (CI:357-553)	109 (CI:65-152)
	Extended	**82 (CI:59-106)**	436 (CI:338-534)	109 (CI:65-153)
Above-ground growth analysis				
2013	Short	**201 (CI:197-205)**	**210 (CI:209-211)**	10.9 (CI:9.9-11.9)
	Extended	**253 (CI:244-264)**	**207 (CI:205-210)**	13.3 (CI:11.5-15.3)
2014	Short	**326 (CI:316-336)**	204 (CI:203-206)	16.3 (CI:15-17.6)
	Extended	**351 (CI:341-363)**	205 (CI:203-207)	17.1 (CI:15.8-18.6)
2018	Short	**237 (CI:231-244)**	**191 (CI:190-193)**	12.6 (CI:11.6-13.7)
	Extended	**286 (CI:280-292)**	**196 (CI:195-197)**	13.7 (CI:12.8-14.8)
2019	Short	316 (CI:307-325)	218 (CI:216-219)	14.6 (CI:13.3-15.9)
	Extended	312 (CI:299-234)	219 (CI:217-222)	15.9 (CI:14.2-17.8)
2020	Short	289 (CI:283-295)	205 (CI:204-207)	12.4 (CI:9.7-14.7)
	Extended	299 (CI:292-307)	206 (CI:205-208)	12.7 (CI:9.5-15.2)

Table S2. *Summary of 'background' root samples taken shortly after maize planting*

Year	Replicate	Short rotation	Extended rotation
		Root Material (kg ha^{-1})	
2019	1	542	740
	2	158	1028
	3	119	248
	4	68	441
	Mean	*222*	*614*
2020	1	2753	281
	2	136	483
	3	182	982
	4	159	2048
	Mean	*807*	*949*

Table S3. *Summary of fixed effect tests on root mass assuming no background root decomposition*

Source	Assuming 0% background root decomposition	Assuming 100% background root decomposition
	p-value	*p-value*
Year	0.12	0.05
Rotation	0.76	0.87
Year x Rotation	0.91	0.15
Depth	0.58	<0.01
Year x Depth	0.31	0.92
Rotation x Depth	**0.01**	**0.07**
Year x Rotation x Depth	0.25	0.76

**DF = Degrees of freedom*

Table S4. *Summary of root mass added contrasts for the extended versus short rotation at each depth; denominator degrees of freedom differ by depth due to missing data*

Depth	Numerator DF*	Denominator DF	F Ratio	p-value

Assuming no background root decomposition				
0-15 cm	1	14.77	6.69	0.02
15-30 cm	1	16.07	0.08	0.78
30-45 cm	1	16.07	0.14	0.71
45-60 cm	1	16.07	0.56	0.47
Assuming 100% background root decomposition				
0-15 cm	1	36.72	5.60	0.02
15-30 cm	1	37.64	1.59	0.21
30-45 cm	1	37.64	0.25	0.62
45-60 cm	1	37.64	0.68	0.41

**DF = Degrees of freedom*

The roots of the rotation effect run deep

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Highlights:

- Maize grain yields in the four-year rotation were 8% higher than in the two-year
- Maize roots extended 11% deeper in the four-year compared with the two-year
- Maize had less 0-15 cm root biomass in the four-year compared with the two-year
- Four-year rotation maize roots were ‘deeper and steeper’ compared with the two-year
- Differences were not explained by physical soil measurements

Abstract

Context or problem

It is well-established that maize (*Zea mays* L.) grown in extended rotations produces higher grain yields compared to maize grown in one- or two-phase rotations, even when nitrogen (N) is not limiting. Understanding the mechanisms driving this phenomenon, commonly referred to as ‘the rotation effect’, is important for designing cropping systems that use land and resources efficiently. Differences in root systems can influence resource acquisition and therefore yield, but it is unknown if such differences play a role in the rotation effect.

Research question

We hypothesized that maize grown in an extended rotation system exhibits a deeper root structure with less biomass investment compared to maize grown in a short rotation, and that these characteristics would be correlated with differences in grain production.

Methods

Using a long-term experiment established in 2001, we measured maize rooting depth across the growing season, root biomass in 15 cm increments from 0-60 cm, and grain yields in the maize phase of two contrasting rotations: a 2-year rotation of maize/soybean (*Glycine max* [L.] Merr) using inorganic sources of nitrogen (N) and maximum tillage depths of 15 cm (hereafter the ‘short rotation’), and a 4-year rotation of maize/soybean/oat (*Avena sativa* L.)-alfalfa (*Medicago sativa* L.)/alfalfa using a mix of organic and inorganic N sources and inversion tillage to 25 cm (hereafter the ‘extended rotation’). Additionally, we measured soil penetration resistance and soil moisture, and performed a growth analysis on aboveground maize biomass.

Results

From 2013-2020, maize grain yields in the extended rotation were equal to or significantly higher than in the short rotation, averaging 8% higher across eight years (11.0 and 10.2 dry Mg ha^{-1}, respectively). In three seasons (2019-2021), the maximum rooting depth of maize in the extended rotation was significantly deeper than in the short rotation by an average of 11% (82 versus 76 cm, respectively), although the timing (e.g., early season, late season) of the extended rotation’s maize growth advantage was not consistent across years. At physiological maturity, the two systems had similar amounts of root biomass from 0-60 cm soil depth, but maize grown in the extended rotation invested significantly less of that biomass (30% compared to 47%) into the soil surface layer (0-15 cm). The soil penetration resistances of the two systems differed in a manner consistent with the differing tillage regimes of the two rotations, however the patterns did not align with root differences.

Conclusions

We posit that the extended rotation's 'deeper and steeper' maize roots did not guarantee higher maize yields, but rather bestowed the plant with more flexibility in resource acquisition which, in certain conditions, resulted in higher grain yields compared to maize grown in the short rotation.

Implications

To our knowledge, this is the first report attempting to mechanistically link rooting patterns with plant growth in the context of the 'rotation effect.' This study enhances our understanding of how cropping system histories impact yields, and provides new data on yields and roots, both of which are highly relevant for sustainable intensification. While the present study focused on physical measurements, it suggests that more detailed exploration of how biological drivers impact root architecture is needed to gain a mechanistic understanding of the 'rotation effect.'

Keywords

Maize; crop rotation, roots, penetration resistance; alfalfa

1 Introduction

Over the past 60 years, the diversity of maize (*Zea mays* L.) production systems in the Midwestern United States (US) has been reduced, moving from multi-species rotations that included small grains and forage legumes to maize monocultures or simple alternations of maize and soybean (*Glycine max* [L.] Merr) (Aguilar et al., 2015; Hijmans et al., 2016; Crossley et al., 2021). Presently, five states in the Midwestern US produce approximately one-sixth of the world's maize and soybean grain (FAO, 2020; USDA National Agricultural Statistics Service 2021), and it follows that in the Midwestern US a significant amount of agricultural land is dedicated to a simplified maize/soybean system (Boryan et al., 2011; USDA National Agricultural Statistics Service Cropland Data Layer, 2021). Several unintended, but nonetheless undesirable outcomes have accompanied this simplification including but not limited to increased rates of soil erosion, increased risk of flooding, and increased risks of nitrate pollution (Hatfield et al., 2009, 2013; Schilling et al., 2010; Jones et al., 2018; Pasley et al., 2021). While cropping system re-diversification may offer avenues for ameliorating many of these issues (Tamburini et al. 2020), there are numerous barriers that currently exist to re-diversifying Midwestern systems (Mortensen and Smith, 2020; Weisberger et al., 2021). Regardless, there is value in understanding the mechanisms that may enable cropping system diversification to contribute to better agricultural outcomes.

Midwestern maize-based systems fall into three main categories: (1) continuous maize systems, wherein maize is grown for two or more consecutive seasons; (2) simple rotated maize systems, wherein maize is rotated with soybean; and (3) extended maize rotations, wherein maize is grown in a rotation with two or more years between maize crops, often including a small grain such as oats *(Avena sativa)* and/or a forage crop such as alfalfa *(Medicago sativa)*. Using these definitions, as maize cropping systems move from monocultures to short rotations or from short to extended rotations, maize yields in the Midwest increase by approximately 10 and 5%, respectively (Crookston et al. 1991; Gentry et al. 2013; Liebman et al., 2008; Davis et al., 2012), and occur even when nitrogen is not limiting (Osterholz et al. 2018; Baum et al. 2023). The former phenomenon is commonly referred to as the ‘continuous maize penalty’, and the latter ‘the rotation effect’. To our knowledge it is unclear whether they are distinct expressions of unique mechanisms, or if they represent a continuum of the same mechanistic underpinnings.

There have been numerous studies in the US Midwest exploring the continuous maize penalty (Dick and Doren, 1985; Peterson et al., 1990; Meese et al., 1991; Crookston et al., 1991; Porter et al., 1997; Varvel, 2000; Stanger and Lauer, 2008; Gentry et al., 2013; Al-Kaisi et al., 2015; Farmaha et al., 2016; Seifert et al., 2017; Vogel and Below, 2018; Bowles et al., 2020). There is evidence it is due, at least in part, to yield suppressive mechanisms in the monoculture system, potentially due to soil biological conditions that may constrain root development and therefore resource capture (Crookston et al., 1988; Johnson et al., 1992; Nickel et al., 1995; Goldstein, 2000). The maize yield advantage accrued from extending short rotations to include small grains and forage legumes has received less attention compared to the continuous maize penalty, but has likewise been well-documented (Liebman et al., 2008; Stanger and Lauer, 2008; Coulter et al., 2011). To our knowledge the mechanisms driving the rotation effect in Midwestern maize systems remain uncertain, partially due to the complex changes in management attendant to extended rotations, rendering it difficult to attribute yield increases to a particular driver.

When above-ground crop products are valued, it is desirable for plants to optimize investments in belowground growth. In nitrogen- or water-limited environments, ‘steep, cheap and deep’ root ideotypes have been identified as the most efficient use of root investments (Lynch, 2013; Tron et al., 2015; Thorup-Kristensen and Kirkegaard 2016; Thorup-Kristensen et al. 2020). It is therefore feasible that maize grown in extended rotations could also benefit from this root architecture ideotype. Many characteristics of extended rotations may promote deeper crop roots. In a long-term cropping systems research experiment in Iowa (Liebman et al., 2008; Davis et al., 2012) researchers have found differences in the vertical distributions of resources, microbial communities, and nutrient cycling activity in soil profiles of simple and complex maize systems (Lazicki et al., 2016; King and Hofmockel, 2017; Osterholz et al., 2018; Poffenbarger et al., 2020; Baldwin-Kordick et al., 2022), all of which might impact, or be impacted by, root architectures. Indeed, Lazicki et al. (2016) found differences in maize root distributions in plots with varying rotation histories, but the data were limited to shallow depths (0-20 cm) and did not control for previous crop root carryover, which may impact interpretations (Hirte et al. 2017).

We hypothesized that maize grown in extended rotations develops a deeper root structure with less biomass investment compared to maize in a short rotation, and that these differences would be correlated with differences in grain production. To test this hypothesis, we made the following measurements in the maize phase of short (maize/soybean) and extended (maize/soybean/oats-alfalfa/alfalfa) rotations:

1. Maize grain yields from eight growing seasons (2013-2020)
2. Maize root biomass from 0-60 cm soil depths as a proxy for the resources invested by the maize crop into roots in two growing seasons (2019-2020)
3. Maximum maize rooting depth as a proxy for the soil space made available for resource capture by that investment in three growing seasons (2018-2020)

Additionally, we complemented these measurements with detailed aboveground maize growth analysis throughout five seasons (2013, 2014, 2018, 2019, 2020); soil penetration resistance measurements in three seasons (2018-2020); and soil moisture and temperature measurements in two seasons (2018-2019).

2 Methods and Materials

2.1 Study location

The experimental site was located at the Iowa State University Marsden Farm, in Boone County, Iowa, USA (42°01' N; 93°47' W; 333 m above sea level). Dominant soil series were Clarion loam, Nicollet loam, and Webster clay loam, all of which are Mollisols. Before the initiation of the experiment, the site had been managed for at least 20 years with a maize-soybean rotation receiving conventional fertilizer and herbicide inputs. Baseline soil samples (0-20 cm) showed a mean buffer pH of 6.8 and mean organic matter concentration of 51 g kg^{-1} (Liebman et al., 2008). The experiment was arranged as a randomized complete block design with each crop phase of each rotation system present every year in four replicate blocks (Payne, 2015). Plots were 18 m by 84 m, with the entire experiment comprising ~9 hectares. Weather data were collected from a weather station approximately one kilometer from the field site (Iowa Environmental Mesonet, 2021).

2.2 Sampling

This study utilized two of the three maize-based rotations present in the larger study: a 2-year rotation of maize/soybean (hereafter the short rotation), and a 4-year rotation of maize/soybean/oat-alfalfa/alfalfa that periodically received composted cattle manure (hereafter the extended rotation). Oat straw and alfalfa were harvested and removed from the research site; no grazing occurred on the plots. Detailed accounts of plot management are reported elsewhere (Liebman et al., 2008; Hunt et al., 2020) and a summary is presented in **Table 1**. Maize grain yield was measured in 2013-2020, and additional measurements were taken in select years during that period (**Table 2**).

Table 1. Summary of agronomic management of the two rotation treatments housing the maize phase (bolded) sampled in this study; for more details see Liebman et al. 2008 and Hunt et al. 2020

Rotation	Crop sequence	Nitrogen sources	Tillage regime
Short 2-year	Soybean/ **Maize**	Mean total of 180 kg ha^{-1} inorganic nitrogen applied to maize phase, with 112 kg ha^{-1} applied at planting and the remaining at V6 side-dressing based on soil nitrate sampling (Sawyer and Mallarino 2017)	Fall chisel plowing and spring field cultivation preceding soybean planting, spring surface cultivation preceding maize planting
Extended 4-year	Soybean/ Oat-Alfalfa/ Alfalfa/ **Maize**	Mean of 140 kg ha^{-1} organic nitrogen applied as composted cattle manure the fall preceding the maize phase, and 32 kg ha^{-1} inorganic N applied at V6 side-dressing based on soil nitrate sampling (Sawyer and Mallarino 2017)	Fall chisel plowing and spring surface cultivation preceding soybean planting, no tillage events preceding oat/alfalfa planting, fall moldboard plowing of the alfalfa crop followed by spring disking and surface cultivation preceding maize planting

2.3 Statistical analysis

Statistical analyses were conducted using *R version 4.0.2* (R Core Team, 2020) with the *tidyverse* suite of packages (Wickham et al., 2019) or JMP Pro 17.0 (SAS Institute, Cary, North

Carolina, USA). Additional R packages were used for specific analyses as described below. Significance thresholds for statistical tests was set at p=0.05 unless noted otherwise. In all cases several statistical models were fit and compared using Akaike's Information Criteria (AIC; Kuha, 2004) and residual plots, but only the model chosen as providing the best fit is reported. All data used in this manuscript are available in csv format and as an R package available for download (https://github.com/vanichols/maRsden). The R code used to perform these analyses and create manuscript figures is available in a public Github repository (will be made public once manuscript is accepted for publication).

2.4 Grain yields, harvest indices, grain weights, and root-to-shoot ratios

The effect of rotation treatment (short, extended) on maize grain yields was assessed using a linear mixed effects model with rotation, year-factor, and their interaction as fixed effects, and block nested within a year-factor as a random intercept using R package *lme4* (Bates et al., 2015). The R packages *emmeans* (Lenth et al., 2018) and *lmerTest* (Kuznetsova et al., 2017) were used for comparisons and statistical summaries of the model, using a Tukey adjustment for multiple comparisons.

Differences in harvest indices, 500-grain weights, and the root-to-shoot ratios were assessed using a linear mixed effects model with rotation, a year-factor, and their interaction as fixed effects and a random block intercept, with a Tukey adjustment for multiple comparisons.

2.5 Rooting depth

We modeled rooting depth as a function of the cumulative maize growing-degree-days (GDDs) accrued since planting (base temperature 10° C, maximum temperature 30° C) to facilitate comparisons between years. Non-linear models were fit using the R package *nlraa* package (Miguez, 2021). We tried several non-linear models fit to both the raw data and the data filtered to remove measurements taken after the season's maximum depth had been reached (**Figure S1**). We found a three-parameter logistic curve (Archontoulis et al., 2015) fit to the filtered data produced the best fit according to AIC and inspection of residuals:

Eqn. 1
$$rootdepth(GDD) = \frac{A_{sym}}{(1+\exp\left(\frac{(x_{mid}-GDD)}{scal}\right))}$$

where *rootdepth(GDD)* is the maximum rooting depth at a given cumulative GDD after planting and A_{sym}, x_{mid}, and *scal* are estimated parameters. A_{sym} represents the maximum rooting depth achieved, x_{mid} represents the cumulative GDD value at which half of the maximum rooting depth was achieved, and *scal* describes the steepness of the curve (Miguez et al., 2018). We found the best model fit incorporated a fixed effect of rotation on A_{sym}, *scal*, and x_{mid}; a random effect of a year-factor on A_{sym}, x_{mid}, and *scal*; a random effect of block on A_{sym}; and a power variance structure (Pinheiro and Bates 2022).

Table 2. Summary of measurements used for this study

Measurement	Years	Description
Maize grain yield	2013-2020	Maize grain yield was determined using a 6-row combine equipped with a yield monitor and moisture meter. Sampling areas for yield were approximately 4.6 m x 84 m and were six rows away from plot edges. All yields are reported on a dry weight basis.
Maize above-ground biomass	2013, 2014, 2018, 2019, 2020	Maize above-ground biomass was measured approximately every two weeks throughout the season. Eight plants were cut at ground level, separated into leaf, stem, and reproductive components, dried at 60° C for at least 72 hours, then weighed.
Maize maximum rooting depth	2018, 2019, 2020	Maximum maize rooting depth was measured starting at 20, 23, and 41 days after planting in 2018, 2019, and 2020, respectively. Sampling was repeated approximately every 2 weeks until 84, 106, and 151 days after planting in 2018, 2019, and 2020, respectively. Maximum rooting depth was determined visually using the protocol of Ordóñez et al. (2018). A soil core was drawn with a 19-mm-diameter soil probe, white roots were identified, and their depth recorded to the nearest cm using a meter stick. At a given date, samples were taken repeatedly from the same core location until no white roots were identified in the core. Four cores were taken per plot at each sampling date.
Soil moisture and temperature at 15 and 45 cm depths	2018, 2019	5TM soil moisture and temperature sensors (METER Group Inc., Pullman, WA, USA) were installed at two depths (15 and 45 cm) in each plot. Data were recorded hourly during the growing season using EM50 data loggers.
Penetration resistance	2018, 2019	Penetration resistance was measured using a FieldScout 900 Soil Compaction Meter (Spectrum Technologies, Inc., Aurora, IL, USA). Values were recorded every 2.54 cm to 45 cm depth immediately following planting and/or approximately 60 days after planting. Ten measurements were taken randomly throughout each plot. Measurements were taken within two days after a saturating rain to avoid capturing differences due to soil moisture.
Maize root mass 0-60 cm	2019, 2020	Maize root mass to a soil depth of 60 cm was measured at 3 and 105 days after planting in 2019, and at 4 and 117 days after planting in 2020. Four soil samples per plot were drawn at a location 10 cm from maize rows in 15 cm depth increments using a 32-mm-diameter soil probe. Soil from each depth increment within a plot was composited and roots were recovered using a sequence of elutriation (washing), flotation, recovery from organic debris using tweezers and a stereo microscope, drying, and weighing (Dietzel et al., 2017).

11

2.6 Root mass

Distinguishing newly added root biomass from the previous year's crop and weed root biomass is difficult using visual sorting, and failure to address these background levels of roots can lead to overestimates of new root mass, with the overestimate varying by cropping history (Hirte et al. 2017). To address this issue, the three main options currently available to field researchers include the use of isotopes, correction for background levels of root material by using a baseline measurement or root growth cores, or maintaining a crop- and weed-free area to track background levels throughout the season. We chose to take a background sample shortly after the maize crop was planted. Additional details of sampling protocols are presented in **Table 2**. When interpreting the data, we used the two extreme assumption cases concerning the percentage of the sampled background roots that decayed between the start of the maize growing season (**Table 2**) and the last sampling date near maize physiological maturity: 0% background root decay, and 100% background root decay. The true amount of background roots present at the time of sampling falls between the extreme assumptions, and we therefore report the production of new crop root biomass as a range of possible values.

The Restricted Maximum Likelihood (REML) method for linear mixed effect models in JMP Pro 17.0 was used to evaluate the effects of rotation treatment on maize root mass production within four soil depth increments (0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm) for each of the extreme assumption cases. Year, rotation system, soil depth, and date were treated as fixed factors, and block and its interactions with the fixed factors were treated as random effects. Sampling depth was nested within year and rotation. Due to the inherently high variability of root measurements, for these analyses the threshold for investigating contrasts (as allowed by degrees of freedom) was set at $p<0.10$.

2.7 Growth analysis

We modeled above-ground biomass as a function of day-of-year using a three-parameter logistic curve (Eqn. 1), as it provided the best fit of all the non-linear models assessed (logistic, Richards, Gompertz, Weibull, Beta; Miguez et al. 2018) based on AIC criteria. A model using GDDs produced similar results, but we chose to report the model using calendar days because it was conceptually easier to compare systems. A separate model was fit for each rotation in each year to allow for derivatives to be taken for each rotation in each year. For visualization purposes, the first- and second-order derivatives of the fitted equation were used to visualize the absolute and relative growth rates over time, allowing for visualization of when differences in growth rates occurred rather than relying on parameter interpretations alone. The effect of rotation on fitted parameters was assessed using the *confint* function of base R.

2.8 Root-to-shoot ratios

A root-to-shoot ratio for each experimental unit was estimated using the range in possible root additions in the 0-60 cm soil profile and the maximum above-ground biomass as predicted by the growth analysis (see Section 2.7). In Iowa, maize roots in the 0-60 cm depth increment account for more than 90% of total root mass (Nichols et al. 2019) but will nonetheless provide an underestimate of the root-to-shoot ratio (Ordóñez et al. 2020). We therefore calculated the ratio to be interpreted as relative, rather than absolute, values.

2.9 Penetration resistance

Penetration resistance was statistically modelled separately for each year and date of sampling using a generalized additive mixed model with a fixed intercept effect of rotation treatment, a fixed 'wiggle' component of rotation treatment, five knots, and a random 'wiggle' effect of block. Generalized additive models can model highly non-linear relationships and are useful when the goal is to compare treatments rather than to create predictions. The *gamm* function of the R package *mgcv* (McCulloch and Neuhaus, 2005; Wood, 2011) was used, and the *emmeans* package was used to assess pairwise comparison significance. Models were fit using both the raw and square-root-transformed data. Although the model on the transformed data produced a better fit according to inspection of residuals, statistical conclusions were not different in the two models so the results from the untransformed data are presented for ease in interpretation.

2.10 Soil moisture and temperature

The hourly soil moisture and temperature data were averaged over 24-hour periods (12:00 am to 11:59 pm) for analysis. The daily means were modelled statistically as a function of day-of-year separately for each year and depth using a generalized additive mixed model with a fixed intercept effect of rotation treatment, a fixed 'wiggle' component of rotation treatment, 35 knots, and a random 'wiggle' effect of block using the R package *mgcv*.

3 Results

3.1 Grain yields and weather

Maize yields ranged from 7.0 to 12.7 dry Mg ha^{-1} over the 2013-2020 study period. The effect of rotation depended on year ($p<0.001$; **Figure 1**), with the maize grown in the extended rotation producing significantly higher ($p<0.01$) grain yields in five (2013, 2014, 2016, 2017, and 2018) of the eight years. Averaged over all years, maize grown in the short rotation yielded a mean of 10.2 dry Mg ha^{-1}, while maize grown in the extended rotation yielded 8% higher with a mean grain yield of 11.0 dry Mg ha^{-1}.

Over the past 30 years, from 15-April through 15-October the research site averaged 597 mm of precipitation with a mean air temperature of 19º C. From 2013-2020, growing season weather conditions varied considerably compared to these long-term averages, with the grain yield dataset capturing conditions in all four temperature/precipitation combinations (**Figure 1**). Years that included growth analyses were likewise represented in all four quadrants, and the three years with full datasets (grain yields, growth analysis, root data) represented all but cool and wet conditions.

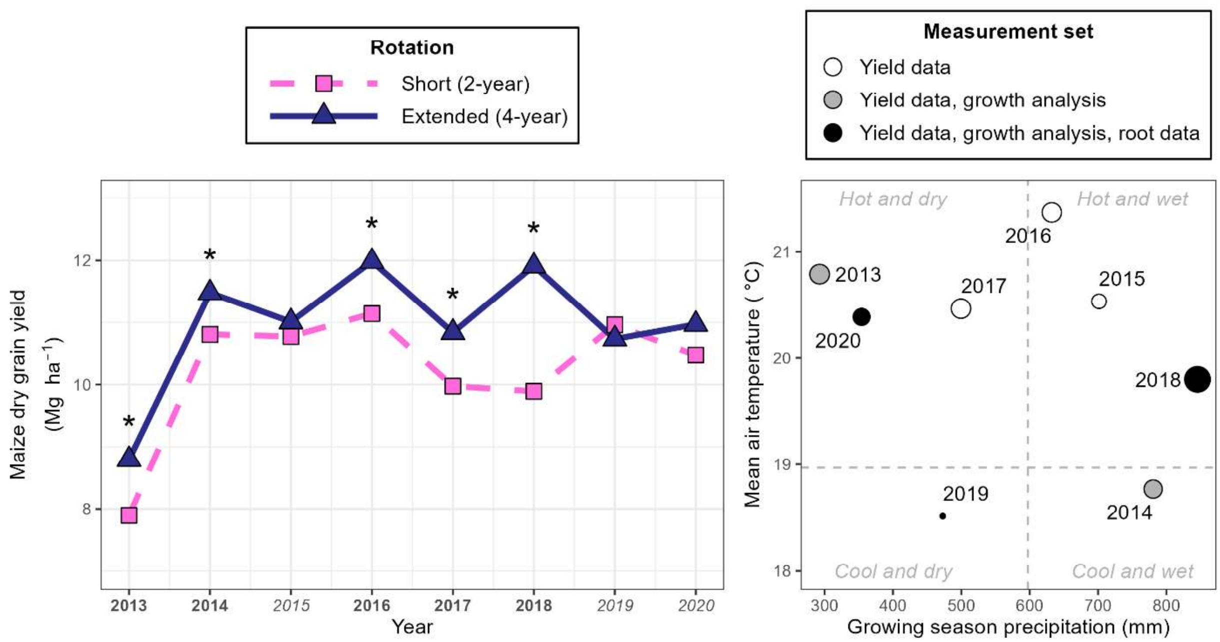
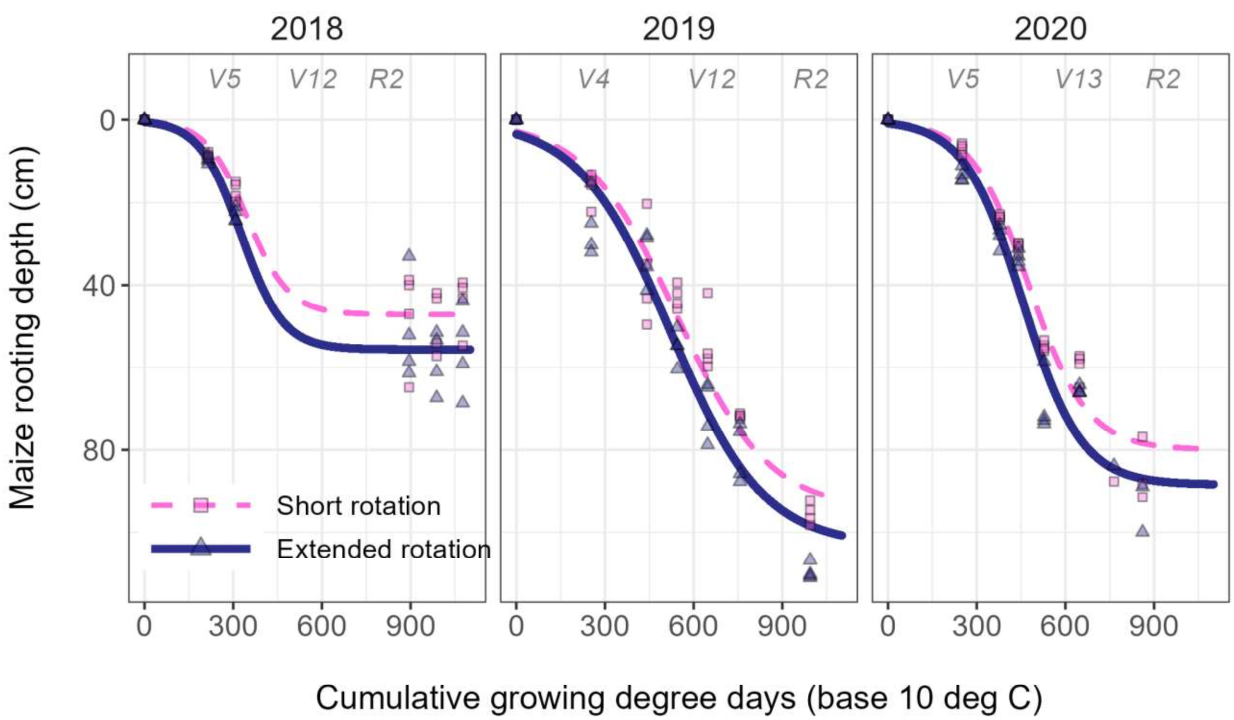


Figure 1. Maize yields and growing season weather; (left) mean maize grain yields (n = 4) in the extended (dark blue, solid, triangles) and short (pink, dashed, squares) rotations from 2013-2020 (lines connecting points for ease in viewing), years with significantly different yields (p<0.01) indicated with asterisks and bolded year font; (right) growing season precipitation and mean air temperatures of each measurement year as compared to 30-year means (dotted lines) with size of points proportional to the extended rotation's maize mean yield advantage over the short rotation, and point color representing the measurement set for that year.

3.2 Rooting depth

The rooting depth of the maize grown in the extended rotation trended consistently deeper in the majority of sampling times in all three growing seasons (**Figure 2**). The maximum rooting depths in 2018 were shallow (~50 cm) due to an extremely wet year (**Figure 1**) that caused consistently shallow water tables as documented at a nearby experimental site (Ebrahimi et al. 2019). Rotation affected maize maximum rooting depth (A_{sym}; $p<0.01$; **Table S1**), estimated at 11% deeper in the extended rotation compared to the short (82 cm and 76 cm, respectively). While the extended rotation roots also descended faster, the effect was not statistically significant (x_{mid}; $p=0.19$).



298

299 *Figure 2. Maize rooting depth across three growing seasons (2018, 2019, 2020; refer to Figure*
300 *1 for weather conditions) in short (pink, dashed, squares) and extended (dark blue, solid,*
301 *triangles) rotations; points represent individual measurements, lines represent the non-linear*
302 *model fits, italic text is the maize stage; model uncertainty is omitted for visual simplicity but can*
303 *be viewed in supplemental material (**Figure S1**); first points at 0 depth indicate maize planting*
304 *date.*

305 ## 3.3 Root mass

306 Root mass present at maize planting (hereafter 'background' root mass) ranged from 68
307 to 2753 kg ha^{-1}, with the extended rotation tending to have higher amounts (**Table S2**). Year
308 interactions were not significant, (**Table S2**), so results are presented as marginal estimates over

years. At the 0-15 cm depth increment, the root mass added in the two systems differed significantly ($p = 0.02$; **Table S3**), regardless of background root decomposition assumptions; the short rotation added between 314 (standard error of the mean [SE]:110) and 506 (SE:56) kg ha^{-1}, while maize grown in the extended rotation added between 101 (SE:110) and 321 (SE:55) kg ha^{-1} of root mass (**Figure 3**). The extended rotation's lower maize biomass relative to the short rotation in the top 15 cm was counterbalanced by higher biomass relative to the short rotation in soil layers deeper than 15 cm (**Figure 3**). Although these differences were not significant (**Table S3**), this pattern resulted in the total root biomass of the two rotations being statistically equivalent. Dietzel et al. (2017) studied maize root growth using in-growth cores and found maize added 480-560 kg ha^{-1} in root material over the growing season in the top 30 cm of soil, suggesting the ranges found in our study are reasonable (**Figure 3**).

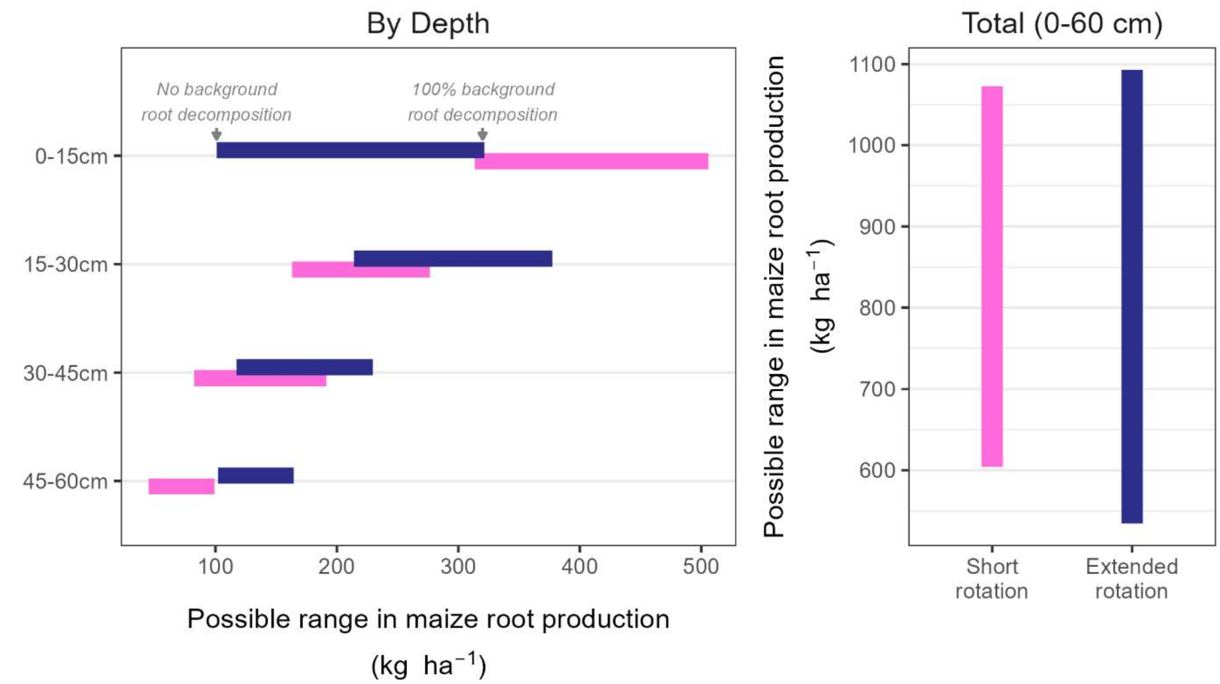


Figure 3. (Left) Possible ranges in mean maize root mass production (n=8) depending on assumed rate of decomposition of root mass present at planting ('background' roots) for maize grown in the short (pink) and extended (dark blue) rotations separated by depth and (right) range in total possible root biomass for 0-60 cm. Data are averages from two growing seasons.

3.4 Growth analysis

The maximum aboveground maize biomass (*Asym*; Eqn. 1) as estimated from the growth analysis was significantly higher in the extended rotation in the years when the extended rotation yielded significantly higher grain yields, but were not different in years without significant differences in grain yields (**Table 3; Figure S2; Table S1**). The date at which the maize achieved half of its maximum biomass (*xmid;* Eqn. 1) was significantly earlier in the extended rotation in 2013 ($p = 0.05$) and exhibited higher absolute growth rates compared to the short rotation before maximum growth rates were achieved (e.g. early in the season; **Figure S2**). Conversely, *xmid* for the extended rotation occurred significantly later than for the short rotation in 2018 ($p < 0.01$) and had higher absolute growth rates after maximum growth rates were achieved (e.g. later in the season). The timings of growth were not significantly different in the other three years with growth data. The maximum growth rates in the two rotations (*scal;* Eqn. 1) were not significantly different in any of the five years. The harvest index and 500-kernel weights were consistently higher in years with large rotation effects on grain yield but did not differ in years that lacked a strong rotation effect (**Table 3**).

The root-to-shoot ratios ranged from 0.002 to 0.015 depending on the assumed background root decomposition (**Figure S3**). Trends were inconclusive due to the variation produced by the assumptions leading to calculation of the ratios. While these root-to-shoot ratios are lower than those reported in literature for maize (e.g., 0.09 from Ordóñez et al. 2020), our root measurement methodology and driving questions differed, and our total root biomasses are consistent with other studies that control for background root amounts (e.g. Dietzel et al. 2017).

Table 3. Summary of characteristics of maize in the extended rotation relative to maize in the short rotation, including grain yields (Figure 1), root characteristics (Figures 2, 3) growth analysis (Figure S2, Table S4), and yield components (Figure S2) for years with growth analysis data, ordered by magnitude of grain yield differentials; ratios of values are presented to simplify visual comparisons of rotations

Year	**Ratio of extended:short**	**Extended rotation's maize maximum**	**Timing of extended rotation's**	**Ratio of extended:short**	**Ratio of extended:short**

	rotation maize grain yield[†]	rooting depth/surface roots relative to short rotation	maize growth advantage[‡]	rotation harvest index	rotation 500-kernal weight
2018	1.20	Deeper/-	Late season	1.08	1.13
2013	1.10	-	Early season	1.04	-
2014	1.06	-	ns	ns	-
2020	ns	Deeper/steeper	ns	ns	ns
2019	ns	Deeper/steeper	ns	ns	ns

[†]Ordered by largest to smallest estimated rotation effect on maize grain yield
[‡]Early season refers to periods before the maximum maize growth rate occurred, late season to periods after
* ns: not significant at p=0.05
- indicates those data were not collected that year

3.5 Penetration resistance

Penetration resistance above 30 cm soil depth was consistently lower in the extended rotation compared with the short rotation, regardless of year or sampling period (planting, late season; **Figure S4**). From 0-30 cm, the extended and short rotations had mean penetration resistances of 0.6 and 0.7 MPa at planting, and 1.1 and 1.5 MPa at late season sampling, respectively, corresponding to a 20% lower penetration resistance in the top 30 cm. From 30 to 45 cm, on average the extended rotation had higher penetration resistance compared to the short by an average of 22% (1.1 MPa/0.9 MPa at planting, and 1.7/1.4 MPa in the late season, respectively).

3.6 Soil moisture and temperature

In both years of measurement, soil moisture at 15 cm depth in the extended rotation was significantly lower than in the short rotation for the first month following planting (**Figure S5**). After that period, the effects of rotation on soil moisture were not consistent across years; in 2018 the short rotation soil was consistently wetter than the extended rotation at both 15 cm and 45 cm depths, but in 2019 there was no difference. In 2018, the extended rotation's lower soil moisture at 15 cm was concomitant with a significantly higher soil temperature compared to the short rotation by ~0.5 deg Celsius, but otherwise showed no consistent trends (**FigureS5**).

4 Discussion

Compared to maize grown in a short rotation, we found maize grown in an extended rotation had consistently deeper maximum rooting depths during three years of measurement in widely varying weather conditions (**Figures 1 and 2**), and a root system that was more evenly distributed from 0-60 cm in two years of measurements (**Figure 3**), corroborating evidence from Lazicki et al. (2016) (**Figure S6**).

The hypothesis that these 'steeper and deeper' root characteristics are a consistent feature of the extended rotation requires further testing, but the data collected in the present study provide support for such a hypothesis. These potentially consistent differences in root characteristics did not, however, significantly enhance grain yields in every year (**Figure 1**). We posit that through changes in physical, chemical, and biological soil properties, the extended rotation provides opportunities for maize roots to grow 'deeper and steeper,' possibly positioning the plant to access resources (i.e., water and nutrients) that may be, or may become, available in deeper soil layers. The feasibility of these interactions is supported by a simulation model that varied root front velocities in maize (and therefore maximum rooting depths); the simulation showed that the impact of deeper rooting on maize yields depended heavily on the year, and that the magnitude of yield impact was comparable to that which we observed (unpublished data; **Figure S7**).

The reasons for the 'steeper and deeper' root architecture are not clear. In a previous study, in which Bay et al. (2021) drew soil from the top 20 cm of plots in each rotation system, sieved and homogenized the soil, packed it into rhizotrons at the same bulk density, and grew maize for 21 days, the investigators found that even in the homogenized soil, maize grown in soil from the extended rotation had a maximum rooting depth ~30% deeper compared to the maize grown in the short rotation soil, as well as significantly thinner but more numerous roots. Working with the same plots as in our study, King and Hofmockel (2017) found microbial biomass was both higher and more evenly distributed in the top 20 cm of the extended rotation soil compared to the short rotation, which, if biological drivers are realized in the field, could be contributing to the differences in root distributions seen in the present study. Results from Bay et al. (2021) and King and Hofmockel (2017), combined with the lack of correlation between root distribution patterns and physical measurements (soil penetration resistance, soil temperature, soil moisture) seen in our field study, provide support for the hypothesis that biological and/or chemical drivers in the soil contributed to root differences between maize in the extended- and short rotations.

Nonetheless, while biological and chemical drivers likely play a role, physical drivers may still be contributing to the 'steeper and deeper' maize roots in the extended rotation in the field. The root legacy of the crop preceding maize (alfalfa in the extended rotation, soybean in the short rotation) might have affected the maize root distributions. One study using mini-rhizotrons observed that when a maize crop followed alfalfa, the maize root distribution closely mimicked the alfalfa root distribution, with 41% of the maize roots following old alfalfa root channels (Rasse et al. 1998). Alfalfa root systems tend to be deeper than those of annual crops (Fan et al. 2016), and even with moldboard plowing (20-25 cm depth) used in our study, there would be intact decaying alfalfa root channels that the maize roots may have followed.

The extended rotation had higher penetration resistances from 30-45 cm depths compared to the short rotation, indicating differences in penetration resistance in bulk soil cannot explain the deeper rooting depths in the extended rotation. Previous work has shown that depth to the water table is associated with differences in maize roots (Nichols et al. 2019), and measured water table heights at this site in 2019 (unpublished data) showed the rotation treatment had no discernible effect on water table dynamics, indicating the differences in root distributions between the treatments were not due to differences in the depth to the water table.

Previous studies have found lower bulk densities and higher particulate organic matter in the extended rotation, which likely reflect the moldboard plowing of the alfalfa crop during the fall prior to maize planting as well as greater particulate organic matter from manure additions (Lazicki et al. 2016; Poffenbarger et al. 2020; Baldwin-Kordick et al., 2022). This is consistent with the lower penetration resistances observed in the present study in the top 30 cm, and likely explains the lower soil moisture observed in the extended rotation at planting. While neither system had absolute resistances high enough to meaningfully impede root penetration, the observed differences in penetration resistance in the surface layers (0.1-0.4 MPa) were of a magnitude that could potentially affect root elongation; a study done with intact soil cores found resistances of only 0.3-0.5 MPa reduced maize seeding root elongation by 50-60% in a sandy loam soil (Bengough and Mullins, 1991).

While the rotation benefit observed in the present study was realized in a range of weather conditions (**Figure 1**), it was large in both the wettest (2018) and driest (2013) years. Many climate change scenarios project increased occurrences of precipitation extremes (Zhang et al. 2013), suggesting extended rotations may play an important role in building cropping system resilience to climate change, and that resilience may be due in part to differences in root architecture.

5 Conclusion

This study provides novel evidence that growing maize in extended rotations can result in changes to maize root structure including maximum rooting depths and vertical distributions. The observed changes in root structure may have affected below-ground resource acquisition by the crop, which may in turn have resulted in higher maize grain yields under certain conditions. Differences in penetration resistance and previous crop root legacies may contribute to altered maize root patterns, but there are likely additional biological and/or chemical drivers that are not well understood in field settings, and would benefit from targeted research. Potential impacts of this research include fine-tuning of crop models to account for cropping history-induced differences in maize rooting depth, investigating if/how genetics interact with extended rotations to further augment the benefits of deeper/steeper rooting in the Midwest, and investigating whether the patterns observed in this study hold in other crops.

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8 Author contributions

All authors contributed to data collection, GN and ML performed data analyses, GN wrote the initial draft of the manuscript, all authors contributed to editing of the final manuscript.

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10 Appendix. Supplementary Material

The following figures are included as supplementary material:

Figure S1 – Maximum rooting depth over time, raw dataset and unsmoothed fitted values

Figure S2 - Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight

Figure S3 – Root to shoot ratios for 2019 and 2020

Figure S4 - Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference

Figure S5 - Soil moisture and temperature at 30 and 45 cm depths in the maize phase of the short and extended rotations

Figure S6 – Data from Lazicki et al. 2016

Figure S7 – Simulated impact of changes in root front velocity on maize yield

The following tables are included as supplementary material:

Table S1 – Summary of above-ground growth-analysis and rooting depth non-linear parameter fits

Table S2 - Summary of 'background' root samples taken shortly after maize planting

Table S3 - Summary of fixed effect tests on root mass

Table S4 - Summary of contrasts for the complex versus short rotation for root mass added at each depth

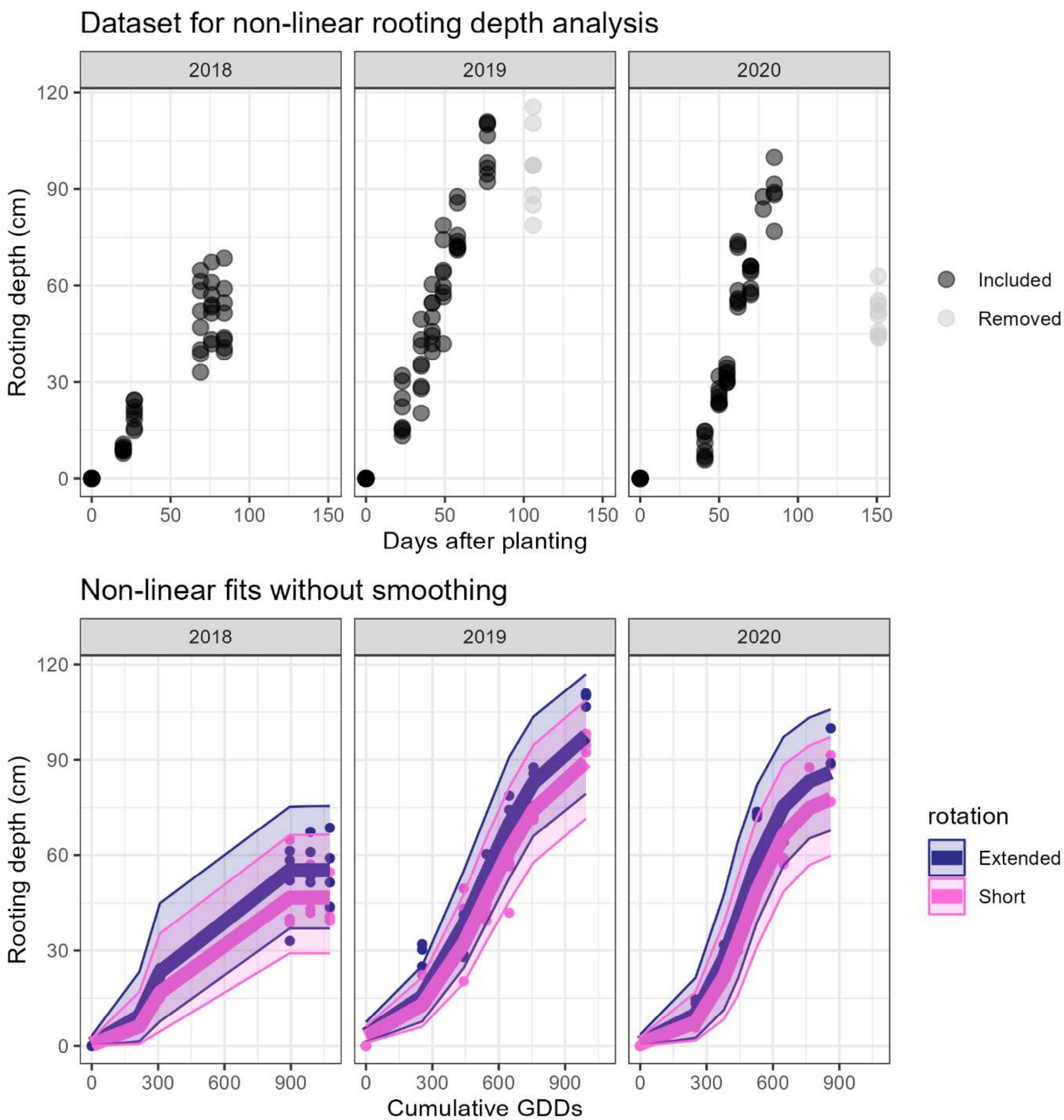


Figure S1 *– Maximum rooting depth over time, raw dataset and unsmoothed fitted values*

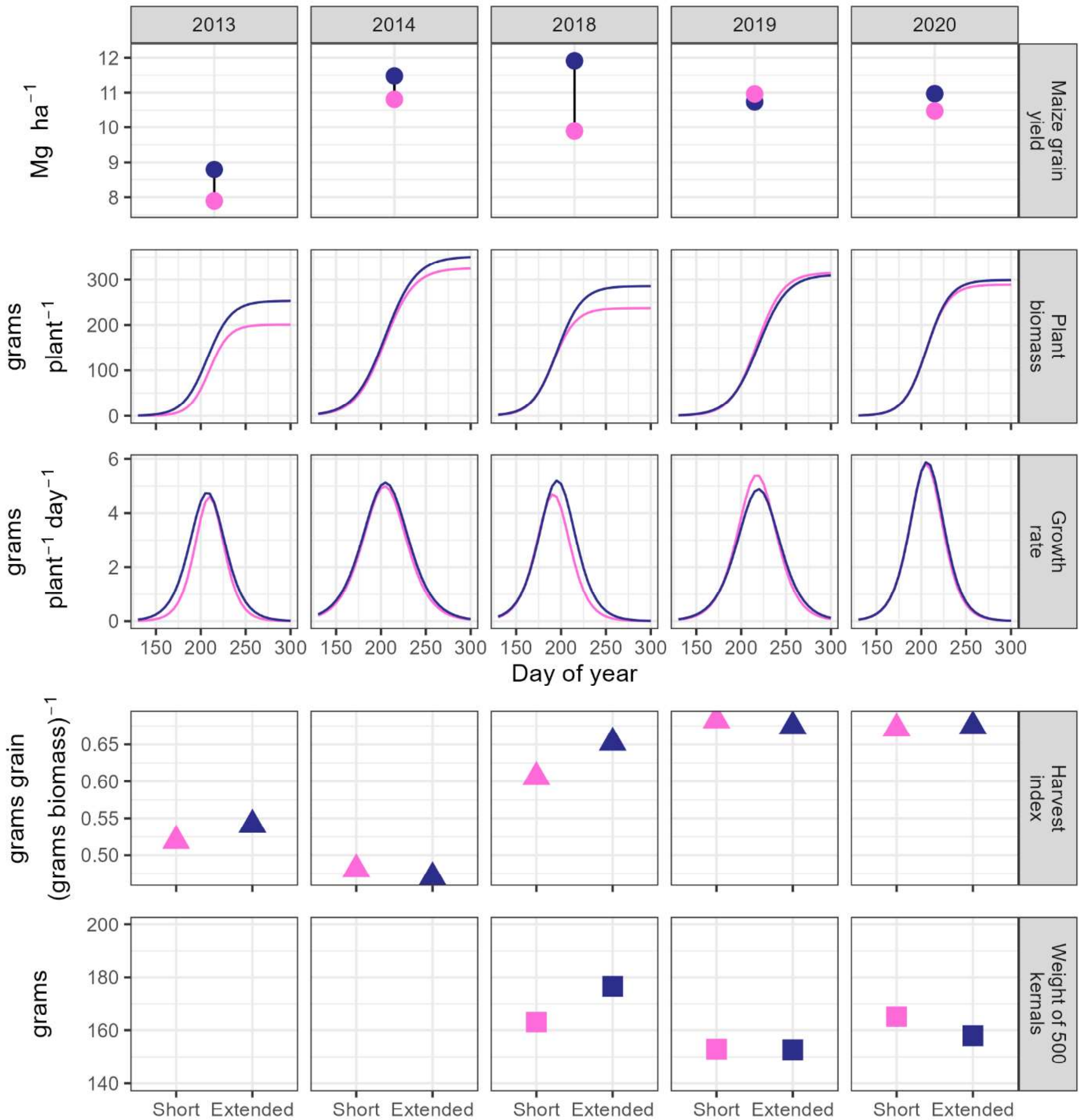


__Figure S2__. Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight

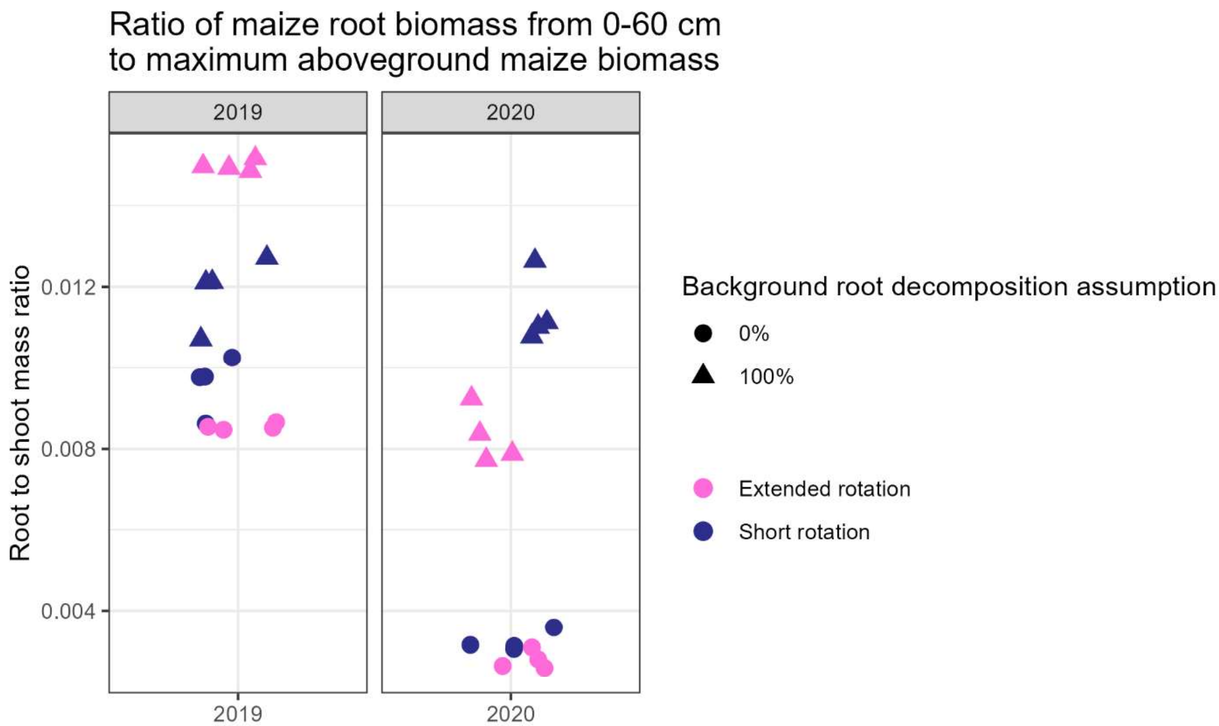
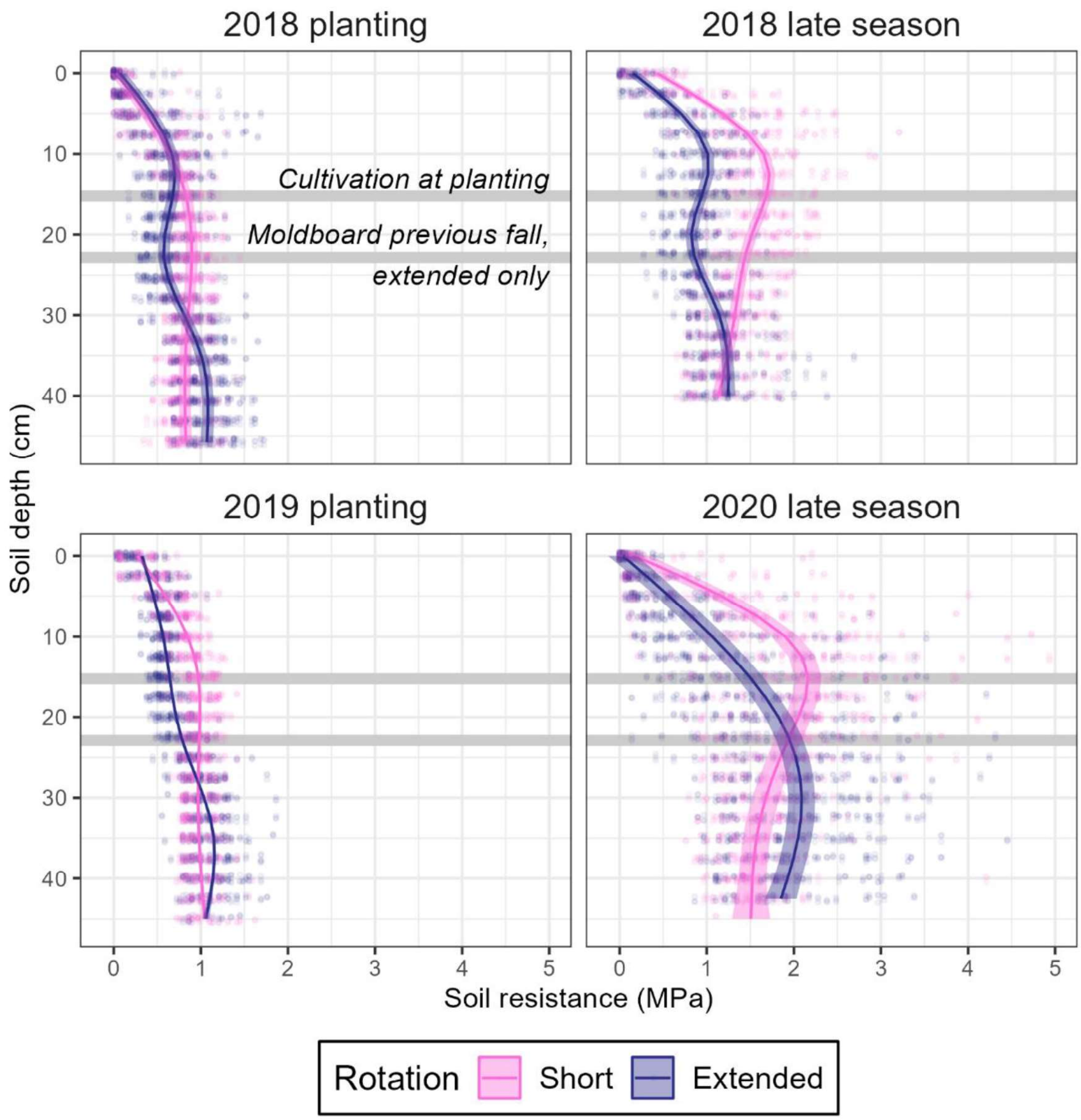


Figure S3. Root to shoot ratios for 2019 and 2020, with different background root decomposition assumptions



717

718 *Figure S4. Soil penetration resistance by depth at various sampling points, approximate depths*
719 *of tillage operations are provided for reference*

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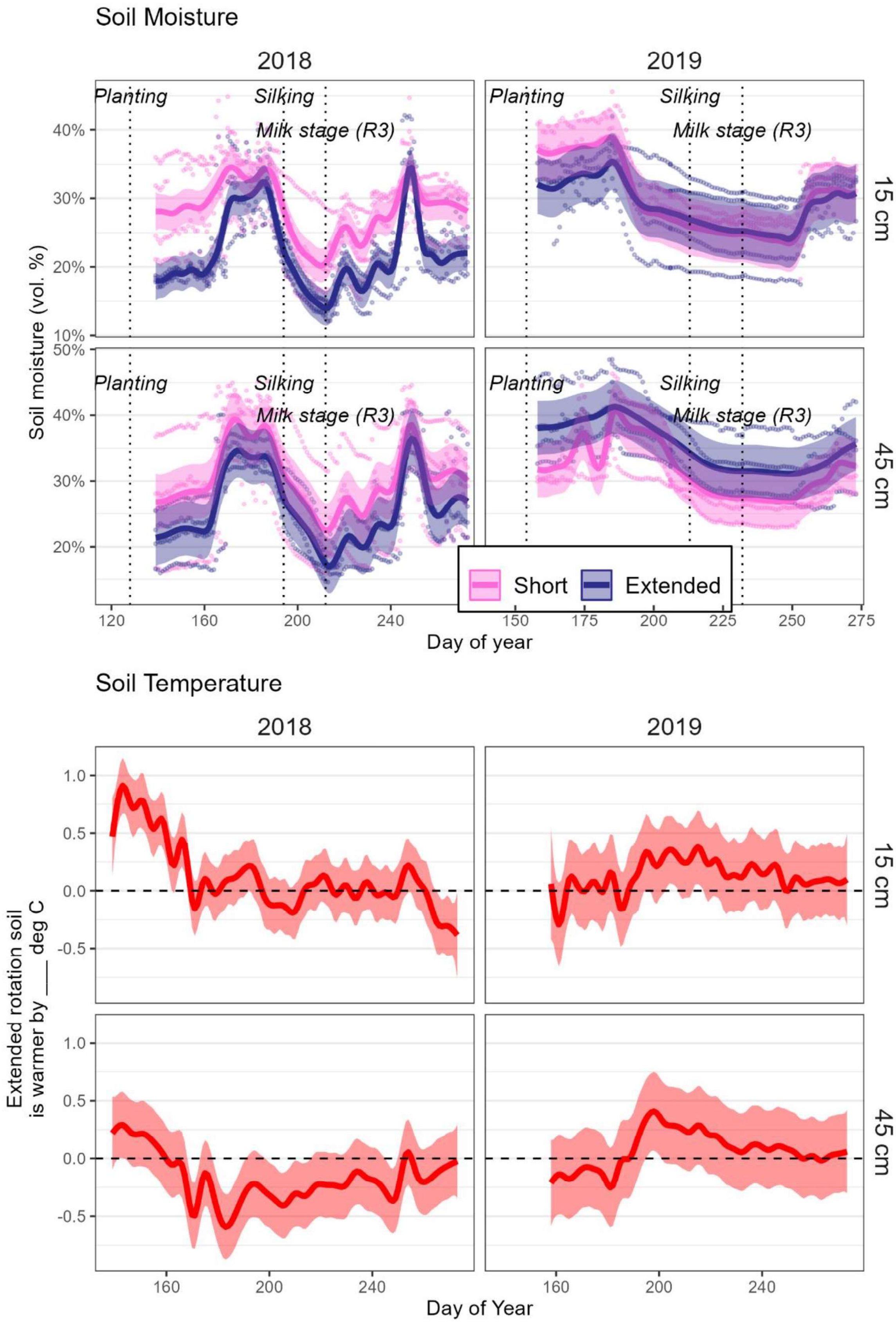

Soil Moisture
2018
2019
Planting
Silking
Milk stage (R3)
15 cm
45 cm
Soil moisture (vol. %)
Short
Extended
Day of year
Soil Temperature
Extended rotation soil is warmer by ___ deg C
Day of Year

721

Figure S5. Soil measurements at 30 and 45 cm depths in the maize phase of the two rotations; (top) soil moisture in the extended (dark blue) and short (pink) rotations, points represent individual sensor values, lines the estimated values, and ribbons the 95% confidence interval around the estimates; (bottom) differences in soil temperature in the extended rotation compared to the short rotation as estimated by GAM with 95% confidence intervals

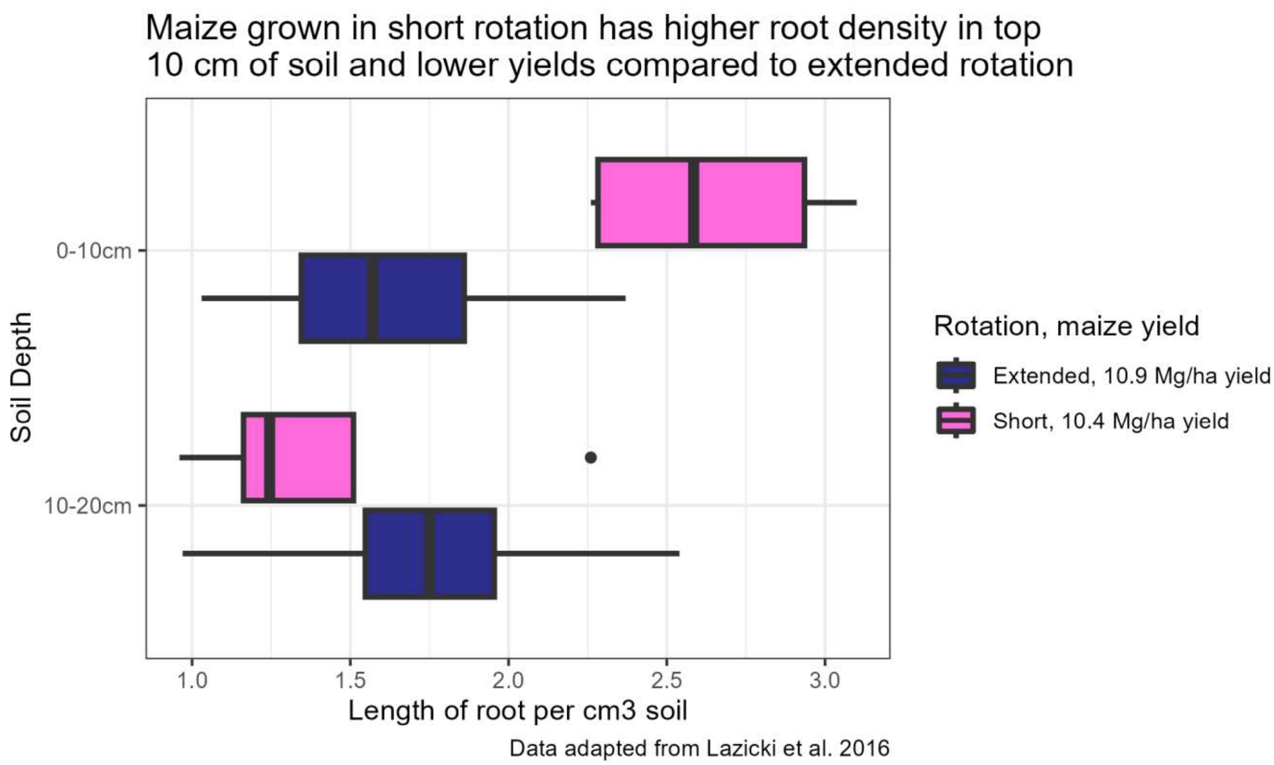

Figure S6 – *Data from Lazicki et al. 2016 shows differences in maize root structures coinciding with maize yield differences in short and extended maize rotations in 2009*

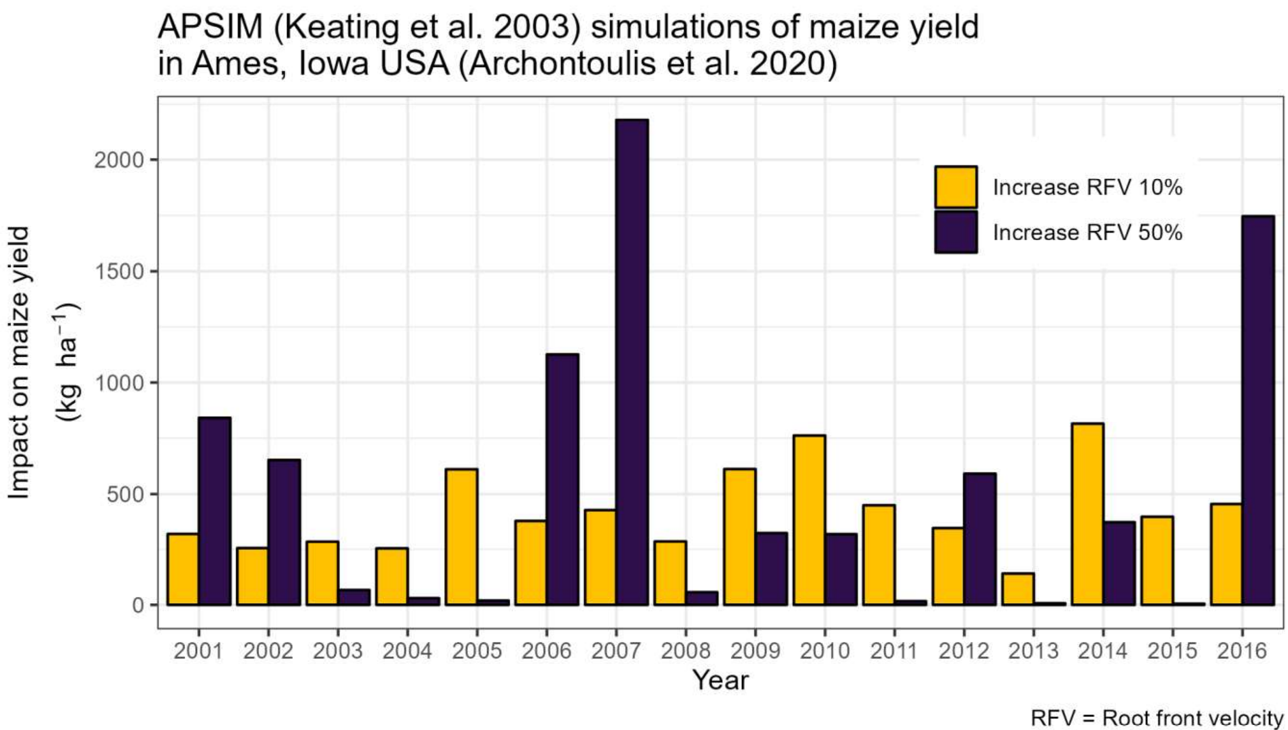

Figure S7 *– Simulated impact of changes in root front velocity on maize yield. Simulations are derived from results supporting the publication of Archontoulis et al. (2020), and the reader is directed to that publication for more details*

Archontoulis SV, Castellano MJ, Licht MA, Nichols V, Baum M, Huber I, Martinez- Feria R, Puntel L, Ordóñez RA, Iqbal J, Wright EE. Predicting crop yields and soil- plant nitrogen dynamics in the US Corn Belt. Crop Science. 2020 Mar;60(2):721-38.

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Table S1. *Summary of 3-parameter logistic curve fits for rooting depth analyses and above-ground growth-analyses, parameters that differed significantly by rotation ($p<0.05$) are bolded.*

Year	**Rotation**	**Asym** *(cm)*	**xmid** *(GDDs)*	**scal**
Rooting depth analysis				
	Short	**74 (CI:50-98)**	455 (CI:357-553)	109 (CI:65-152)
	Extended	**82 (CI:59-106)**	436 (CI:338-534)	109 (CI:65-153)
Above-ground growth analysis				
2013	Short	**201 (CI:197-205)**	**210 (CI:209-211)**	10.9 (CI:9.9-11.9)
	Extended	**253 (CI:244-264)**	**207 (CI:205-210)**	13.3 (CI:11.5-15.3)
2014	Short	**326 (CI:316-336)**	204 (CI:203-206)	16.3 (CI:15-17.6)
	Extended	**351 (CI:341-363)**	205 (CI:203-207)	17.1 (CI:15.8-18.6)
2018	Short	**237 (CI:231-244)**	**191 (CI:190-193)**	12.6 (CI:11.6-13.7)
	Extended	**286 (CI:280-292)**	**196 (CI:195-197)**	13.7 (CI:12.8-14.8)
2019	Short	316 (CI:307-325)	218 (CI:216-219)	14.6 (CI:13.3-15.9)
	Extended	312 (CI:299-234)	219 (CI:217-222)	15.9 (CI:14.2-17.8)
2020	Short	289 (CI:283-295)	205 (CI:204-207)	12.4 (CI:9.7-14.7)
	Extended	299 (CI:292-307)	206 (CI:205-208)	12.7 (CI:9.5-15.2)

Table S2. *Summary of 'background' root samples taken shortly after maize planting*

Year	**Replicate**	**Short rotation**	**Extended rotation**
		Root Material (kg ha^{-1})	
2019	1	542	740
	2	158	1028
	3	119	248
	4	68	441
	Mean	*222*	*614*
2020	1	2753	281
	2	136	483
	3	182	982
	4	159	2048
	Mean	*807*	*949*

Table S3. *Summary of fixed effect tests on root mass assuming no background root decomposition*

Source	**Assuming 0% background root decomposition**	**Assuming 100% background root decomposition**
	p-value	*p-value*
Year	0.12	0.05
Rotation	0.76	0.87
Year x Rotation	0.91	0.15
Depth	0.58	<0.01
Year x Depth	0.31	0.92
Rotation x Depth	**0.01**	**0.07**
Year x Rotation x Depth	0.25	0.76

**DF = Degrees of freedom*

Table S4. *Summary of root mass added contrasts for the extended versus short rotation at each depth; denominator degrees of freedom differ by depth due to missing data*

Depth	**Numerator DF***	**Denominator DF**	**F Ratio**	**p-value**

Assuming no background root decomposition				
0-15 cm	1	14.77	6.69	0.02
15-30 cm	1	16.07	0.08	0.78
30-45 cm	1	16.07	0.14	0.71
45-60 cm	1	16.07	0.56	0.47
Assuming 100% background root decomposition				
0-15 cm	1	36.72	5.60	0.02
15-30 cm	1	37.64	1.59	0.21
30-45 cm	1	37.64	0.25	0.62
45-60 cm	1	37.64	0.68	0.41

**DF = Degrees of freedom*