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 very 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. This lends confidence 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, there is not a significant difference in total root biomass, only the way it is distributed in the soil. We added text to clarify that.

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.  
Again the reviewer is correct – by using the ‘extreme assumptions’ ranges we address this issue. The differences at the deeper soil depths were sensitive to the assumed decomposition, so we do not claim differences in the 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.

It is true there may have been true differences 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 regards 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.

*Nichols, Virginia A., Raziel A. Ordóñez, Emily E. Wright, Michael J. Castellano, Matt Liebman, Jerry L. Hatfield, Matt Helmers, and Sotirios V. Archontoulis. "Maize root distributions strongly associated with water tables in Iowa, USA." Plant and Soil 444 (2019): 225-238.*

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’ 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. 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 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 true. It’s more that the roots are responding to something and that is resulting in different root structures which appear to offer advantages in certain conditions. NEEDS WORK  
  
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.  
2. In table 3, it's unclear how the timing of maize growth advantage is determined/calculated. 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.  
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.  
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.  
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).  
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.  
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.  
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.  
  
  
  
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.  
  
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.  
  
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 applies strongly to references to papers where it is not clear if the complex  
rotation is identical to the one reported here. Value-neutral names of the systems  
would be two-course and four-course rotations. 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.  
  
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.  
  
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.  
  
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.  
  
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 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.  
  
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.  
  
Detail  
  
Report the statistical significance of the higher yield, rooting depth, root biomass and  
penetration resistance in the Abstract and Highlights.  
  
The Conclusions (L258) that the result is 'novel' is inconsistent with many papers  
referred to in the manuscript.  
  
The renumbering of lines makes it difficult to review the manuscript. Please stick  
to one system in any resubmission.  
  
The references are reported in several different formats and should be standardised.