Reviewer comments:    
  
Reviewer #1: Summary: The authors investigate the rotation effect by comparing a short rotation (corn, soy) to an extended rotation (corn, soy, oat-alfalfa, alfalfa). Previous research demonstrated an increase in yield in the extended rotation compared to the short rotation. Differences in resource distribution, microbial activity, and nutrient cycling have previously been observed as well. In this study, the authors focus on maize root growth and its implication on yield. Maize yield is shown to be significantly higher in the extended rotation compared to the short rotation in 5 of the 8 years during this study. Root biomass data suggests maize grown in the extended rotation allocates more roots below 15 cm and increased rooting depth earlier in the season in some years when compared to maize grown in the short rotation. Measurements of soil penetration resistance and soil moisture do not indicate that differences in soil physical properties lead to maize in extended rotation growing more deep roots. The authors suggest that soil biological properties as studied previously may contribute to these differences.  
  
Comments:  
**1. The authors do a good job addressing my previous comments and concerns.**  
Thank you.

**2. I believe there is a typo on line 598; the publication year is in parentheses.**  
This was fixed.

Reviewer #3:  
**This manuscript emphasizes that the whole extended rotation was responsible for the yield benefit and depth of root system of maize, rather than one or more specific features of the rotation. There is some acknowledgement that specific drivers may have contributed to the results (L102-105 and L441-445) but only as secondary drivers to the general effects of extended rotation. More emphasis should be given to the possible effects of the specific drivers.**

The focus of this study was on quantifying the response of the roots to the system, not to specific drivers. As the reviewer points out, we do discuss how individual characteristics of the extended rotation may impact roots (in addition to the reviewer’s references we also discuss individual drivers throughout the discussion). However, we feel that putting too much emphasis on individual drivers in this rotation discounts the complex interactions that are present. While individual characteristics such as manure application, tillage intensity, and legacies of deep-rooted crops such as alfalfa have all been studied in isolation and certainly each may contribute to changes in maize yields, we feel it is important to emphasize how the system is manifesting rather than simplify it to the sum of its individual parts. In L103-105 we directly acknowledge this as an inherent difficulty in studying the impacts of complex rotations.  **It also emphasizes resource acquisition rather than resource supply (e.g.L381). Could the supply of mineralized N in the subsoil arise from the fine-root residues of alfalfa in the subsoil? If so, the proliferation of maize roots in the subsoil may reflect supply of mineral N that preceded its acquisition. Is there evidence of this in Midwest systems?**

We added a conceptual supplementary figure (Figure S7) and the following text to clarify our hypothesis to interested readers.

*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, which may have provided biopores and additional nutrients from alfalfa root decay (Shahzad et al. 2018). Our study suggests consistent differences in the resource acquisition hardware (e.g., roots) in the extended rotation, so while an increased resource supply in the subsoil resulting from alfalfa legacies is certainly possible, it likely varies both spatially and temporally, depending on the root distributions (and growing conditions) of the previous alfalfa crop, as well as the subsoil conditions during the maize growing season. For example, in 2018 the soil below 40 cm was saturated for a large proportion of the growing season, which would likely inhibit alfalfa root decay and therefore limit its contributions to subsoil mineral nitrogen supply that year, but differences in maize roots between the two rotations were none-the-less observed. The consistency of the root characteristics suggest they are not responding to differences in resource availability per se, but rather that they impact a system’s ability to use resources (Figure S7).* **How representative are the rotations and yield responses in this manuscript of previous reports? At L92-95, 13 papers report the continuous maize penalty and 3 papers at L101-102 report extended rotations that include small grains and legumes. Both responses are mentioned in 4 papers at L87&88. Rather than just referring to these papers, the authors should present a table showing the actual rotations and their effect on maize yield in the previous studies and in a form comparable with the presentation of their own data e.g. M S O/A A - 8%. Comparing the cropping patterns and yield responses in previous papers with the pattern in the current manuscript could help to identify mechanisms and drivers.**

We added a citation to Bennett et al. 2012, which summarizes the phenomena of yield declines in short rotations from a global scale, and the following text.

*The impact of extending rotations on crop yields is well-known and has been summarized previously, with reported maize yield increases ranging from 7-36% (Bennett et al. 2012). However this global range covers disparate contexts, with varying flavors of diversification not suited to all production contexts.*

**The manuscript refers to extended rotations in many Midwest studies and the (singular) system reported in this manuscript. When reporting and discussing the results the manuscript should refer to the experiment, not the region's extended rotation.**

This point was addressed with the above addition, and several small additions to contextualize the statements, as the reviewer suggests.  **At ~L40, add to the sentence indicating that the extended rotation significantly (P<0.01) outyielded the short rotation in 5 of the 8 years.**Due to word limits in the abstract, we do not feel the addition of a p-value is needed in this instance. The majority of scientists will interpret the term ‘significantly higher/greater’ as a p-value less than 0.05, and indicating a p-value of 0.01 would not drastically impact the interpretation of this statement.

**The reference to Sawyer and Mallarino. 2017, cited in Table 1 is not available on the web. Related papers report a soil-nitrate sampling depth of 30 cm. Please confirm the sampling depth and report the nitrate concentrations. Are there data from this or related studies on the mineral N amount between the sampling depth and the bottom of the root zone?**

We are unsure why the reviewer is not able to access the reference, which is a widely-downloaded extension publication and the PDF is available for download at the cited link as of 5 July 2024. However, we added the sampling depth (30 cm) to the table to ensure readers have access to that information. The spring soil-nitrate concentrations for the 8 years of this experiment were used for determining the amount of nitrogen applied at side-dress at maize stage V6 in an effort to equalize nitrogen availability in all treatments, and we do not feel these data provide information relevant to this study. The detailed management separated by year is presented in the cited studies (Liebman et al. 2008; Hunt et al. 2020). Moreover, previous studies have shown nitrogen supply in the top 30 cm (the depth to which the late-spring nitrate sampling is done) does not explain the maize yield differences in the short and extended rotation (Osterholz et al. 2018, cited as such in L91 and L118). Based on this and a previous comment, we understand the reviewer’s point that nitrogen supply in deeper layers could be a factor in driving root growth, and have addressed that through the addition of a conceptual figure (Figure S7) and additional text in the discussion.  **Section 3.5: Please indicate whether the measurement of penetration resistance avoided wheel tracks and whether the short-rotation treatments incurred less compaction from vehicle movements than the long-rotation.**

We added the following text to section 2.9:

*Ten measurements were randomly taken within a plot, with two being taken in areas that experienced wheel traffic within the past year. The short rotation saw approximately 6.5 tractor passes per year, while the extended rotation saw approximately 7.5 tractor passes per year. Previous studies have shown tillage significantly reduces the impact of wheel traffic on soil compaction (Voorhees et al. 1983), and the two systems in the present study were tilled to a depth of at least 15 cm. We saw no difference in the wheel-traffic area measurements and bulk plot measurements; we therefore did not include that factor in the statistical model.*

**Why the difference in experimental seasons between Fig. 1 (6 years) and Fig. S2 (5 years)?**

Figure 1 shows eight years of yield and weather (not six as indicated by the reviewer). Figure S2 presents data from the five years where data *in addition* to yield was collected.  **All differences in yield and other properties should be accompanied by a report of statistical significance. This applies specially to the Abstract and Highlights. In parts of the manuscript before the Statistical analysis section the response should be accompanied by a simple indicator such as (P=0.03). Within the Statistical analysis section, add a sentence indicating that responses reported in the paper have e.g. p<0.05.**

In the body of the manuscript we fully agree, and it was our intention to include all p-values. To our knowledge we have; without specific lines where we failed to do so we cannot make the requested corrections. In the highlights, we do not believe including p-values is a good use of word count, and find numerous examples in previously published manuscripts in Field Crops Research that do not include p-values in their highlights. In the abstract we use the word ‘significantly’ when referring to the statistically significant differences in yields (L38), maximum rooting depth (L41), and amount of root biomass (L45). In the manuscript, L171 states ‘Significance thresholds for statistical tests were set at p=0.05 unless noted otherwise’.   
**Fig S7 reports maize yields of 1-2 t/ha. Please correct.**

We appreciate the thoroughness of the reviewer’s comments. The y axis is labeled ‘impact on maize yield’ - it is the change in maize yield, which is correctly presented as 1-2 t/ha. We changed the axis title to ‘increase in maize yield’ to clarify this point.