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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 other resources efficiently. Differences in root systems can influence crop 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 periodic 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% greater across eight years (11.0 and 10.2 dry Mg ha-1, respectively). Although the timing (e.g., early season, late season) of the extended rotation's maize growth advantage was not consistent across years, in all three seasons of root measurements (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),. 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 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.
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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?
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17 July, 2024

To the Editorial Board of Field Crops Research:

On behalf of all co-authors, herewith I am submitting a newly 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 the requested minor changes to the manuscript.

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 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.

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Thank you.

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whether the short-rotation treatments incurred less compaction from vehicle movements than the long-rotation.

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- Maize had less shallow (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 other resources

efficiently. Differences in root systems can influence crop 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 periodic 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% greater across eight years (11.0 and 10.2 dry Mg ha^{-1}, respectively). Although the timing (e.g., early season, late season) of the extended rotation’s maize growth advantage was not consistent across years, in all three seasons of root measurements (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),. 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

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Keywords

Maize; crop rotation, roots, penetration resistance; alfalfa

1 Introduction

Over the past 60 years, consistent with global trends towards agricultural simplification, 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, reduced biodiversity, and increased risks of nitrate pollution (Hatfield et al., 2009, 2013; Hirsh et al. 2013; Berges et al. 2010; 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 diversified cropping systems to contribute to better environmental outcomes from agricultural systems.

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. 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’.

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 in the Midwest 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 short and extended 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 0-30 cm 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 0-30 cm 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 were 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 (https://github.com/vanichols/ghproj_marsden). We ask that users of the data and/or code cite the present study.

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

The effect of rotation treatment 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 10ºC, maximum temperature 30ºC) 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 60ºC 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 (**Figure S2**), 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

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. 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 19ºC. 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.

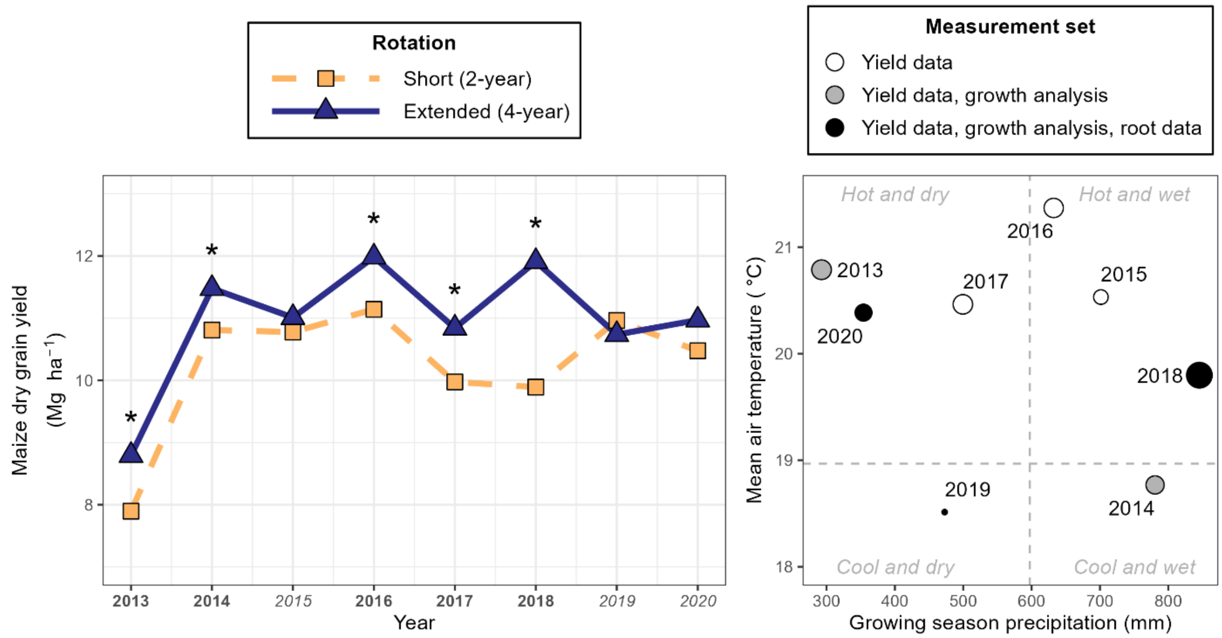

Figure 1. Maize yields and growing season weather; (left) mean maize grain yields (n = 4) in the extended (dark blue, solid, triangles) and short (yellow, dashed, squares) rotations from 2013-2020 (lines connecting points for ease in viewing), years with significantly different yields (p<0.01) indicated 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 for 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).

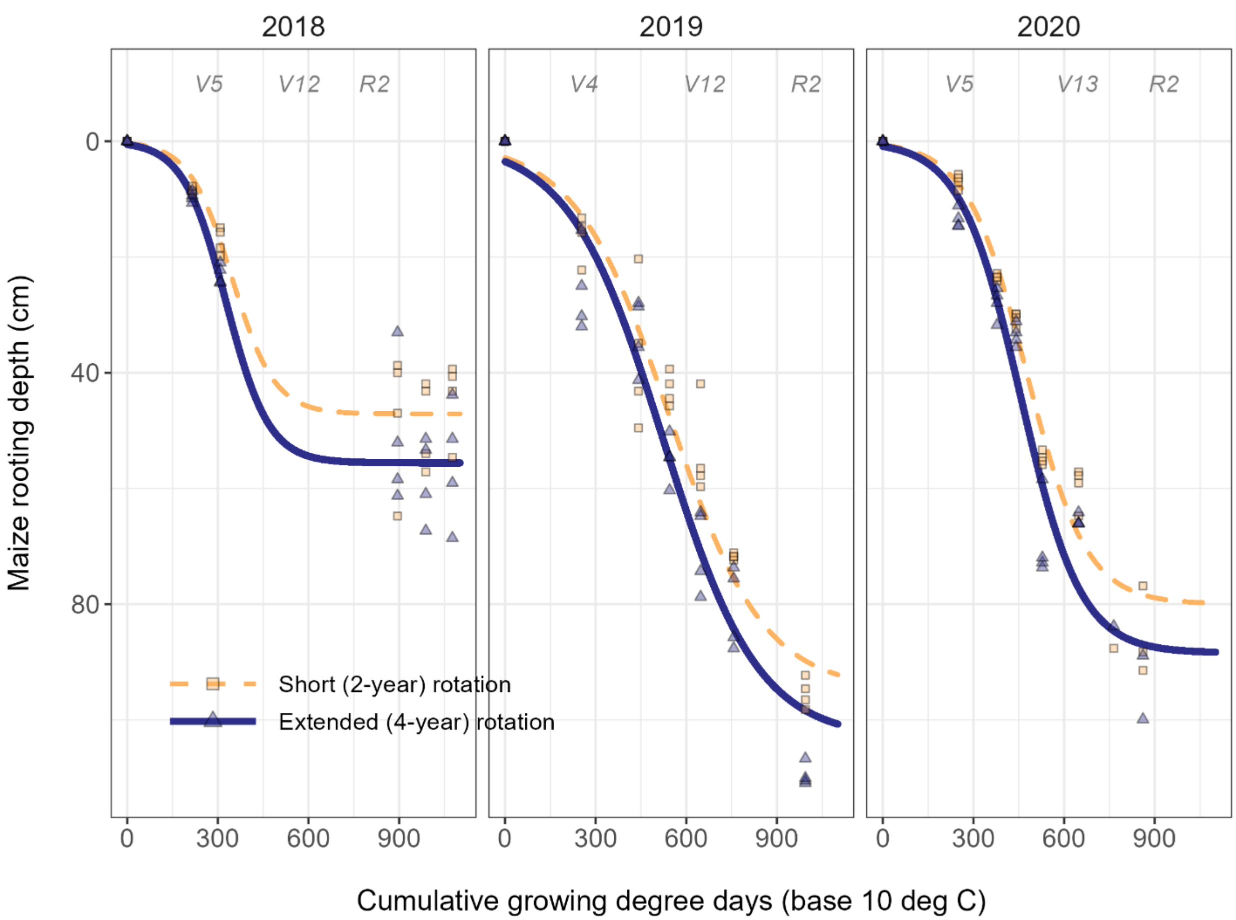

*Figure 2. Maize rooting depth across three growing seasons (2018, 2019, 2020; refer to Figure 1 for weather conditions) in short (yellow, dashed, squares) and extended (dark blue, solid, triangles) rotations; points represent individual measurements, lines represent the non-linear model fits, italic 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 greater 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) quantified 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**).

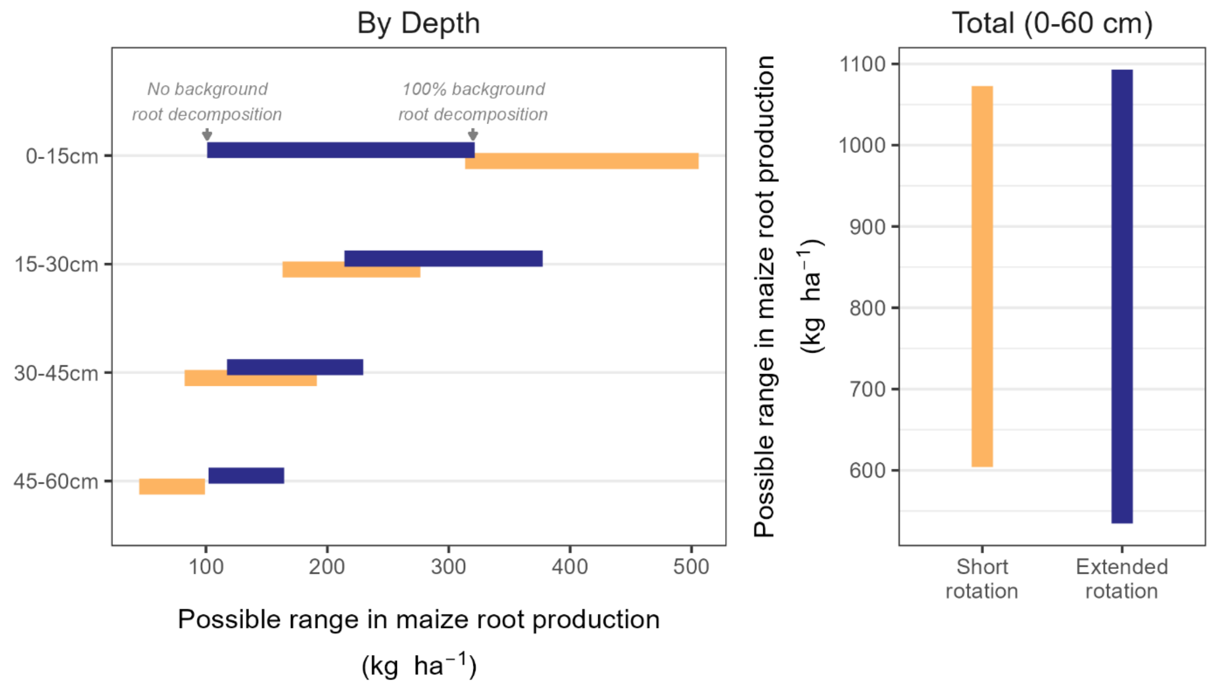

Figure 3. (Left) Possible ranges in mean maize root mass production (n=8) depending on assumed rate of decomposition of root mass present at planting ('background' roots) for maize grown in the short (yellow) and extended (dark blue) rotations separated by depth and (right) range in total possible root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

3.4 Growth analysis

In the years with a significant yield differential between the two rotations, the maximum aboveground maize biomass (*Asym*; Eqn. 1) in the extended rotation was significantly greater than in the short. However, in years where the two rotations' yields were not significantly different, the two rotation's maximum aboveground biomass was likewise not significantly different (**Table 3; Figure S2; Table S1**). In 2013 (a year with significant yield differences), the date at which maize achieved half of its maximum biomass (*xmid;* Eqn. 1) was significantly earlier in the extended rotation ($p = 0.05$) 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, in 2018 (another year with significant yield differences), *xmid* for the extended rotation occurred significantly *later* than for the short rotation ($p < 0.01$) 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 (2014, 2019, 2020). 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 rotation maize grain yield†	Extended rotation's maize maximum rooting depth/surface roots relative to short rotation	Timing of extended rotation's maize growth advantage‡	Ratio of extended:short rotation harvest index	Ratio of extended:short 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**). 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 of the extended rotation soil. 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°C, but soil temperature and moisture 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**). 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,' positioning the plant to access resources (i.e., water and nutrients) that may be, or may become, available in deeper soil layers (van der Bom et al. 2020). 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 hypothesis that ‘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.

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 (Han et al. 2015). 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, 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**).

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 is consistent with 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). Regardless, the patterns in penetration resistance are not consistent with the differences in roots observed in the present study.

While the rotation benefit observed in the present study was realized in a range of weather conditions (**Figure 1**), the rotation benefit 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 and a conceptual figure of our hypothesis

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

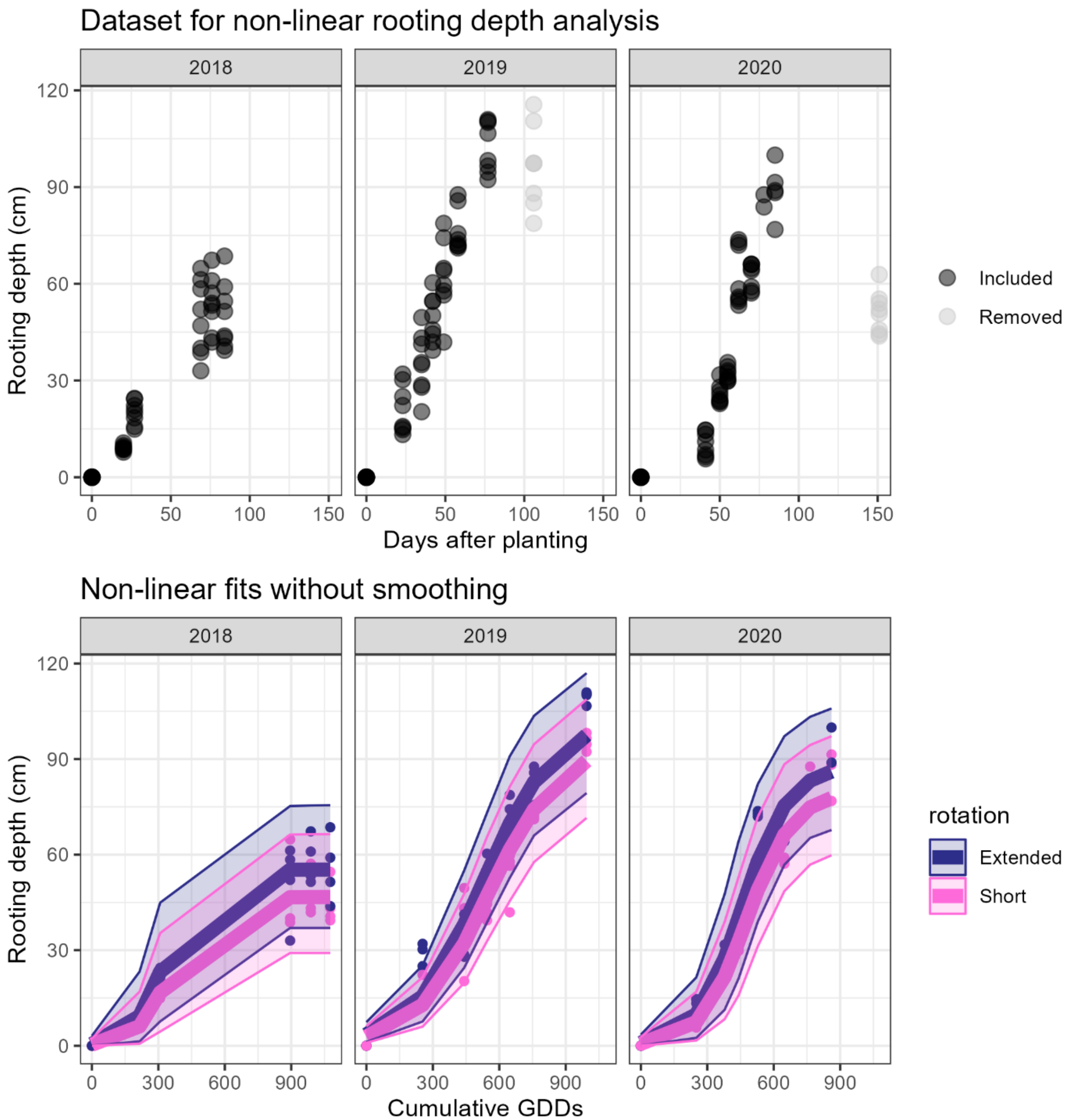


Figure S1 *– Maximum rooting depth over time, raw dataset and unsmoothed fitted values.*

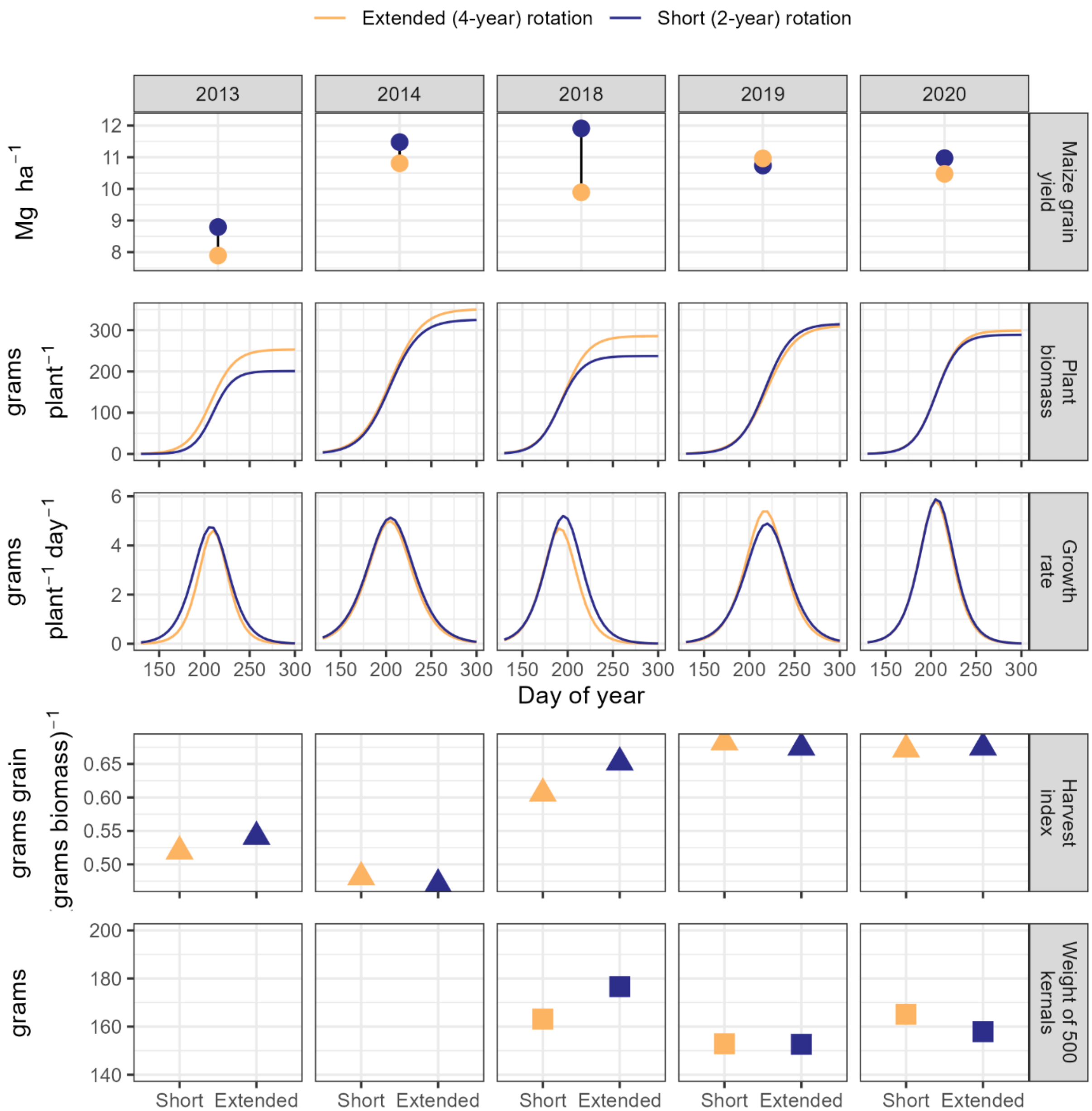


Figure S2*. Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight for the extended 4-year rotation (dark blue) and short 2-year rotation (yellow). The reader is directed to the manuscript text for indications of significant differences.*

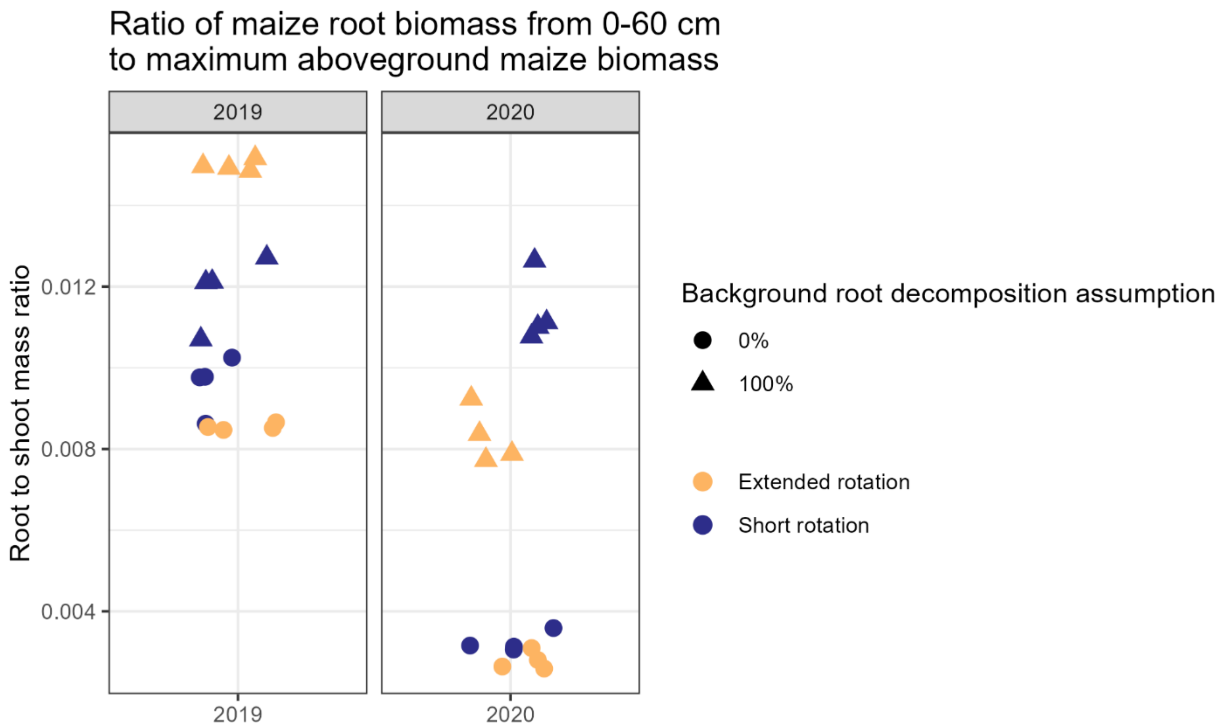


Figure S3. Root to shoot ratios for 2019 and 2020, with different background root decomposition assumptions.

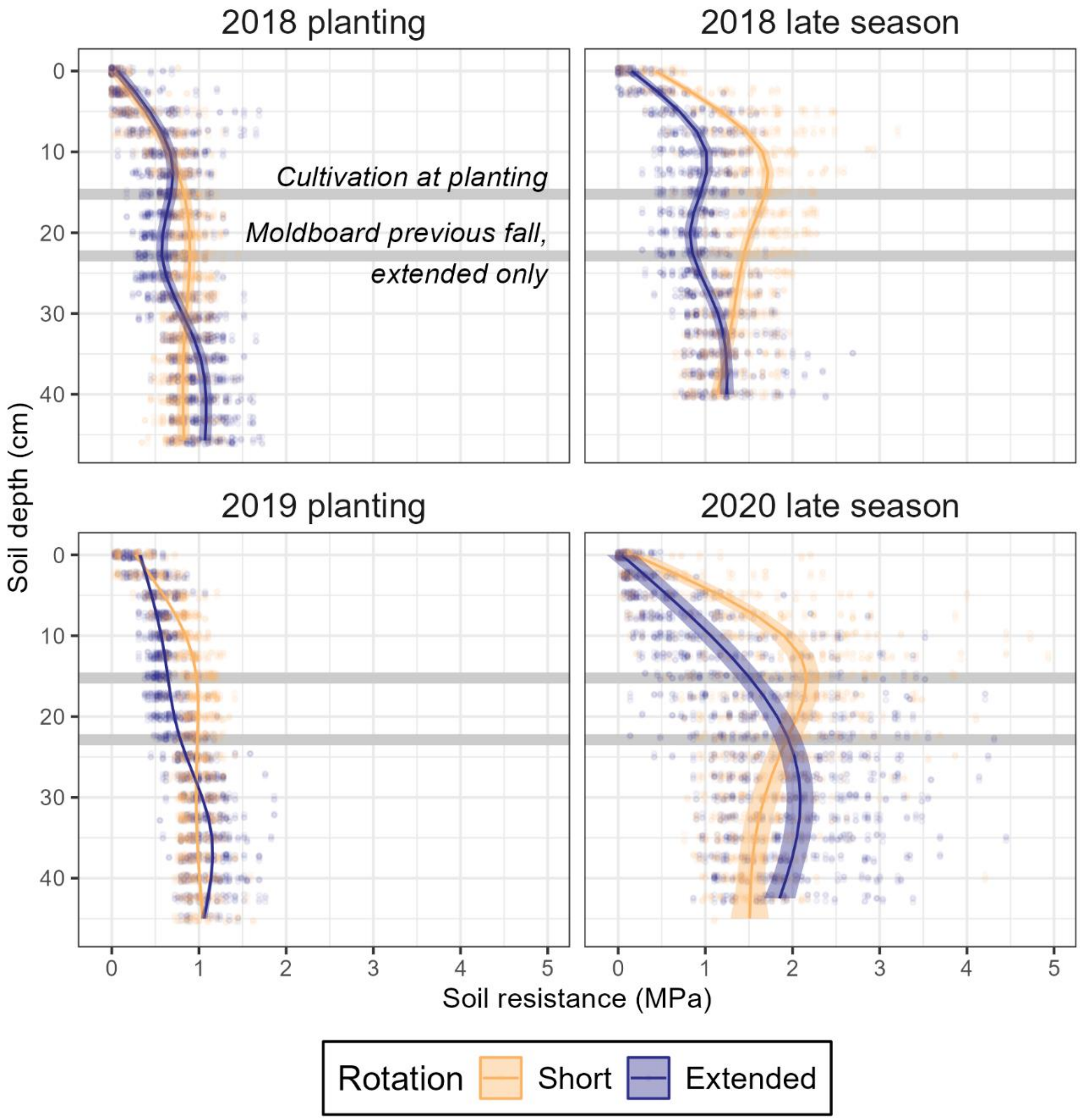


Figure S4. Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference.

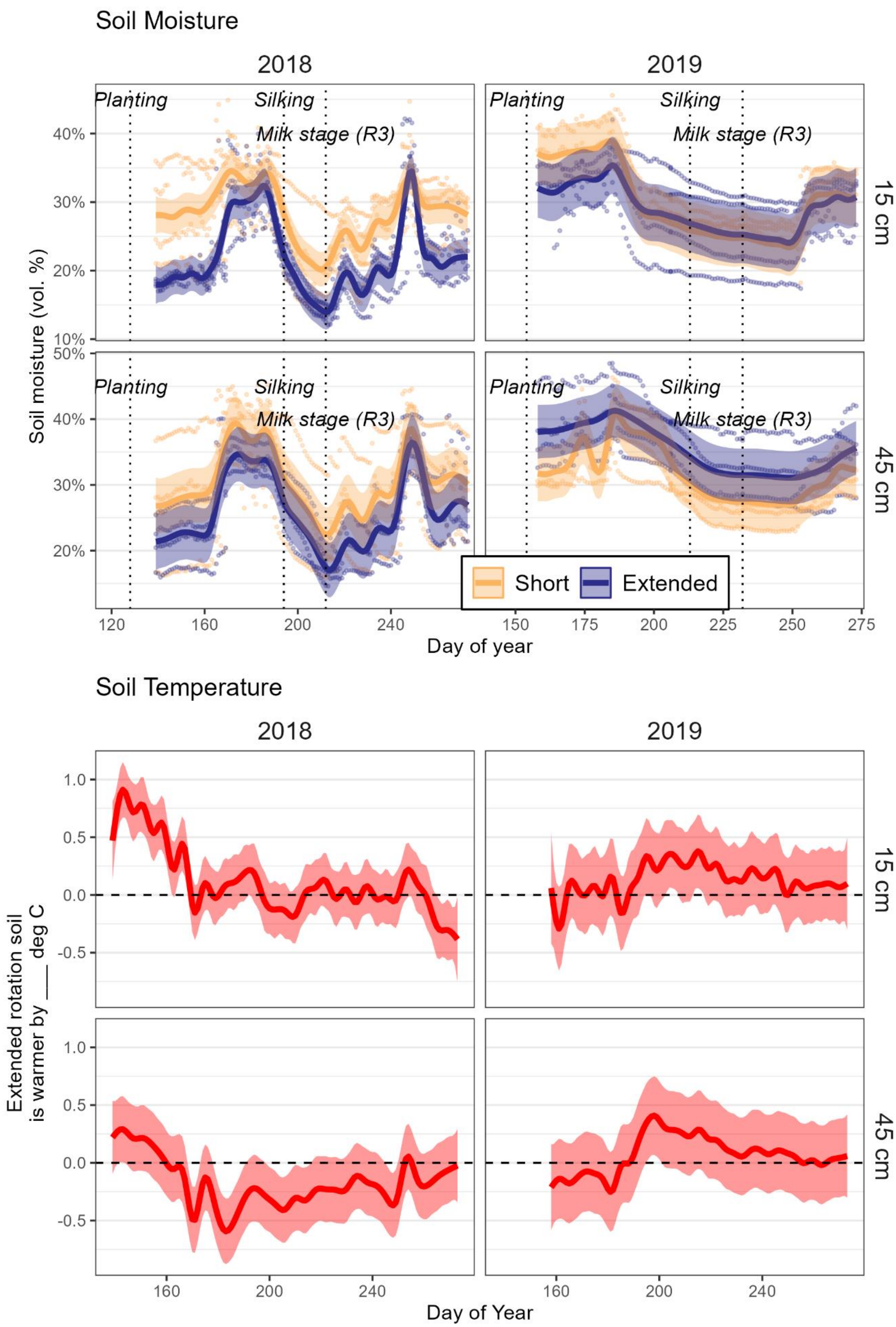

Soil Moisture
2018
2019
Planting
Silking
Milk stage (R3)
Soil moisture (vol. %)
40%
30%
20%
10%
50%
15 cm
45 cm
Short
Extended
120
160
200
240
150
175
200
225
250
275
Day of year
Soil Temperature
Extended rotation soil is warmer by ___ deg C
1.0
0.5
0.0
-0.5
Day of Year

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 (yellow) 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.

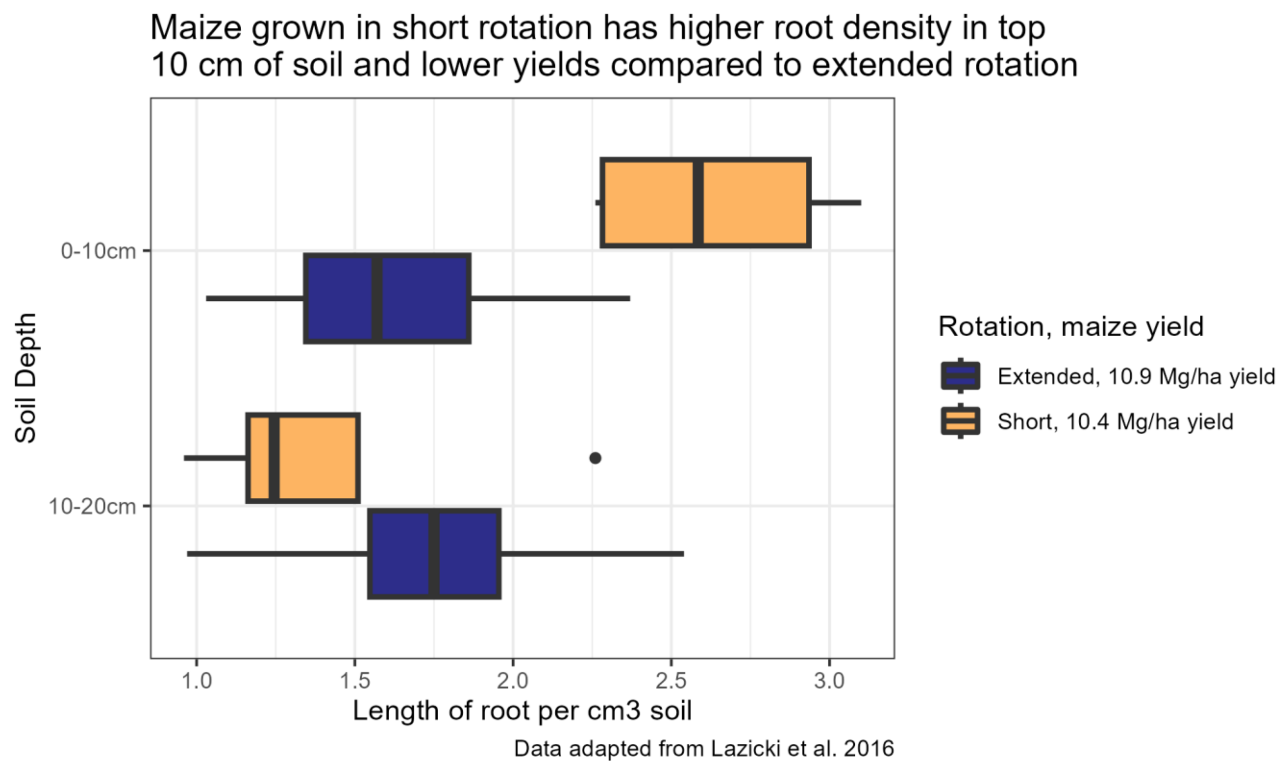
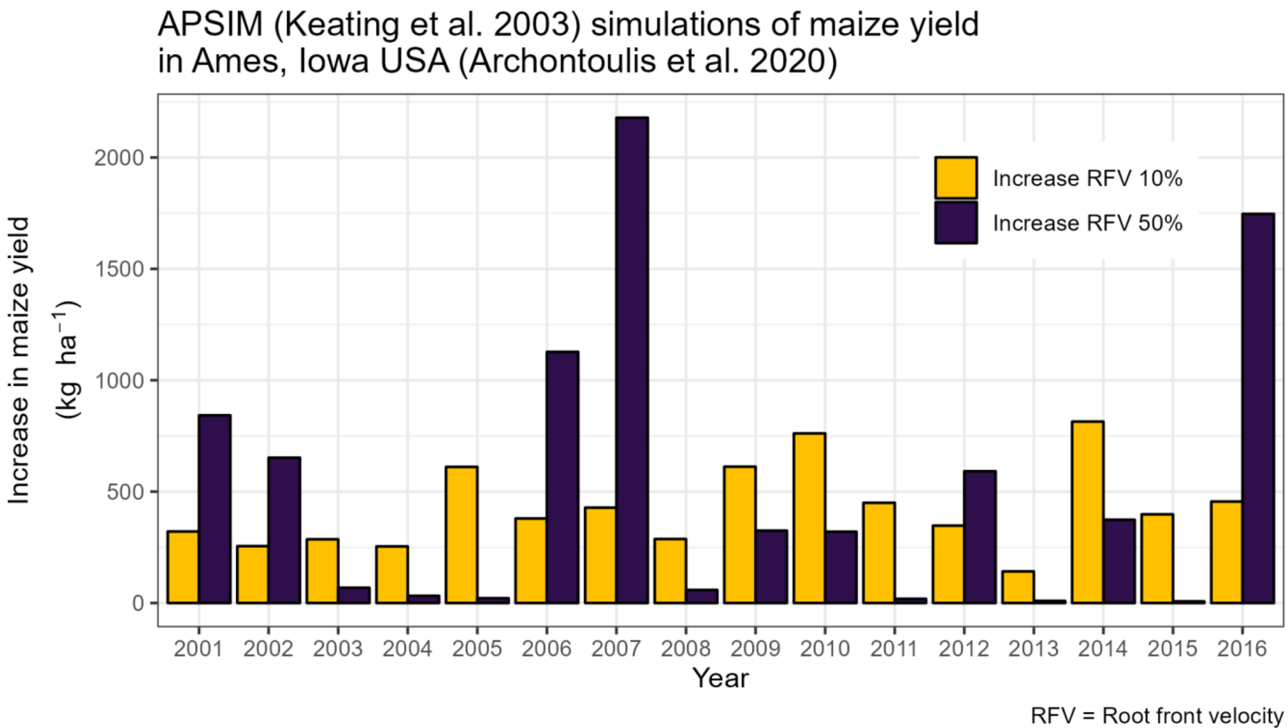


Figure S6 *– Data from Lazicki et al. 2016 shows differences in maize root structures coinciding with maize yield differences in short and extended maize rotations in 2009.*


APSIM (Keating et al. 2003) simulations of maize yield
in Ames, Iowa USA (Archontoulis et al. 2020)
Increase in maize yield
(kg ha^{-1})
2000
1500
1000
500
0
Increase RFV 10%
Increase RFV 50%
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Year
RFV = Root front velocity

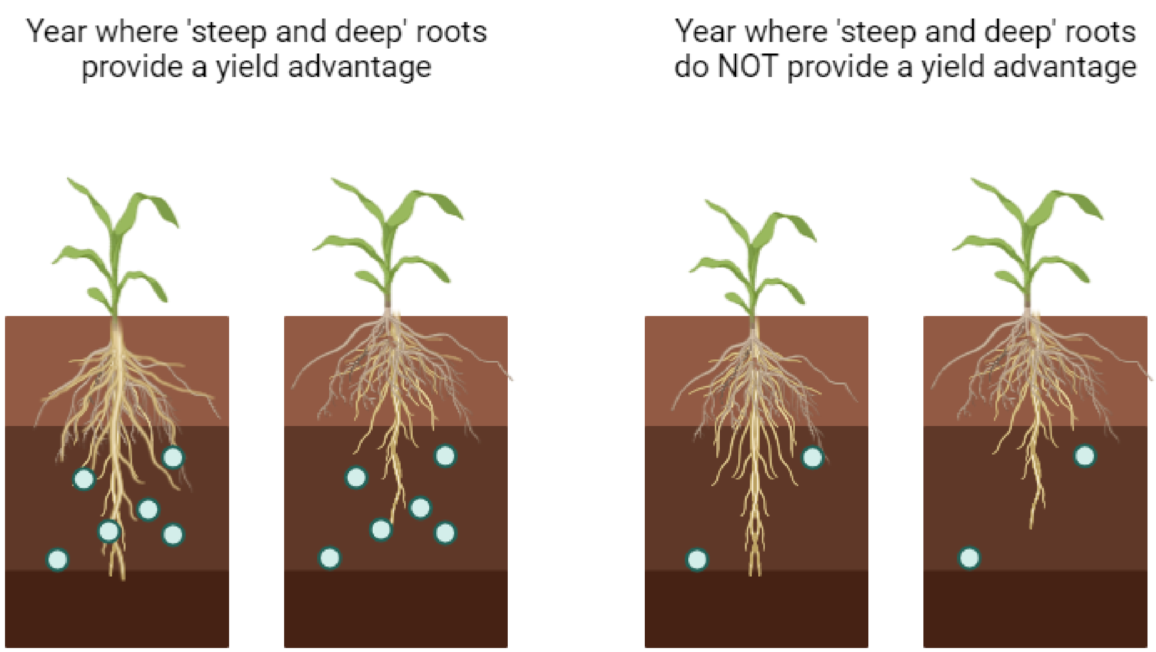

Year where 'steep and deep' roots
provide a yield advantage
Year where 'steep and deep' roots
do NOT provide a yield advantage

Figure S7* – *(Top) *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.* ***(Bottom)*** *Conceptual figure.*

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 shallow (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 other resources efficiently. Differences in root systems can influence crop 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 periodic 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% greater across eight years (11.0 and 10.2 dry Mg ha^{-1}, respectively). Although the timing (e.g., early season, late season) of the extended rotation's maize growth advantage was not consistent across years, in all three seasons of root measurements (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),. 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.

<u>Conclusions</u>

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.

<u>Implications</u>

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, consistent with global trends towards agricultural simplification, 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, reduced biodiversity, and increased risks of nitrate pollution (Hatfield et al., 2009, 2013; Hirsh et al. 2013; Berges et al. 2010; 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 diversified cropping systems to contribute to better environmental outcomes from agricultural systems.

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. 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’.

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 in the Midwest 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 short and extended 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 0-30 cm 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 0-30 cm 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 were 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 (https://github.com/vanichols/ghproj_marsden). We ask that users of the data and/or code cite the present study.

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

The effect of rotation treatment 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 10ºC, maximum temperature 30ºC) 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 60ºC 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 (**Figure S2**), 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

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. 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 19ºC. 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.

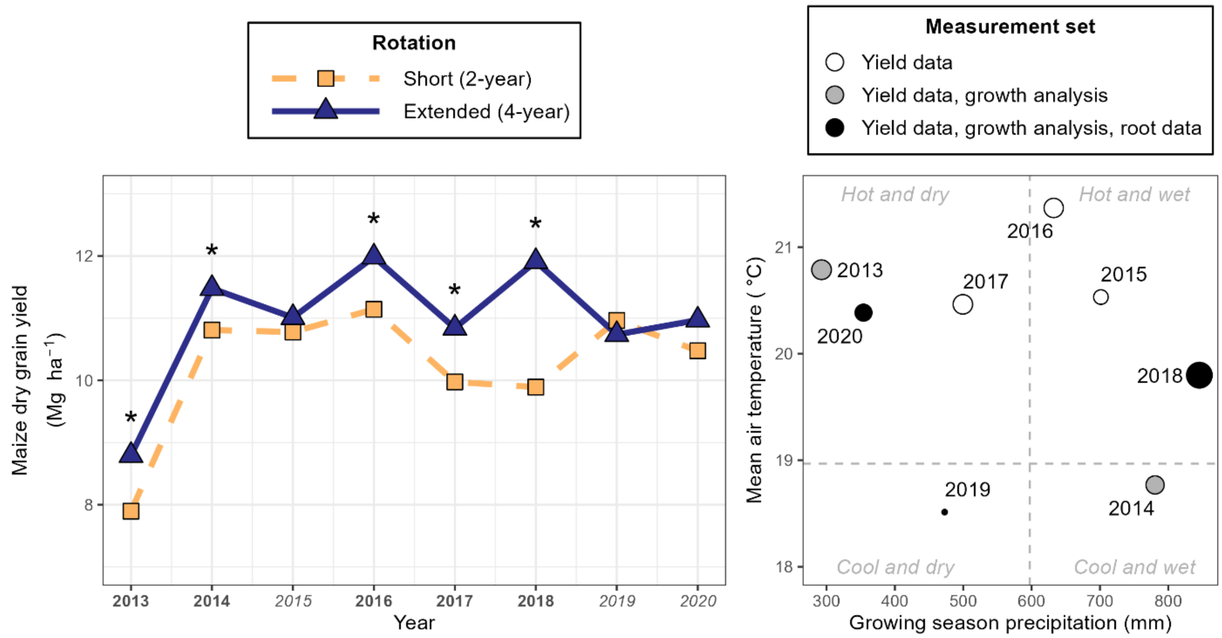

Figure 1. Maize yields and growing season weather; (left) mean maize grain yields (n = 4) in the extended (dark blue, solid, triangles) and short (yellow, dashed, squares) rotations from 2013-2020 (lines connecting points for ease in viewing), years with significantly different yields (p<0.01) indicated 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 for 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).

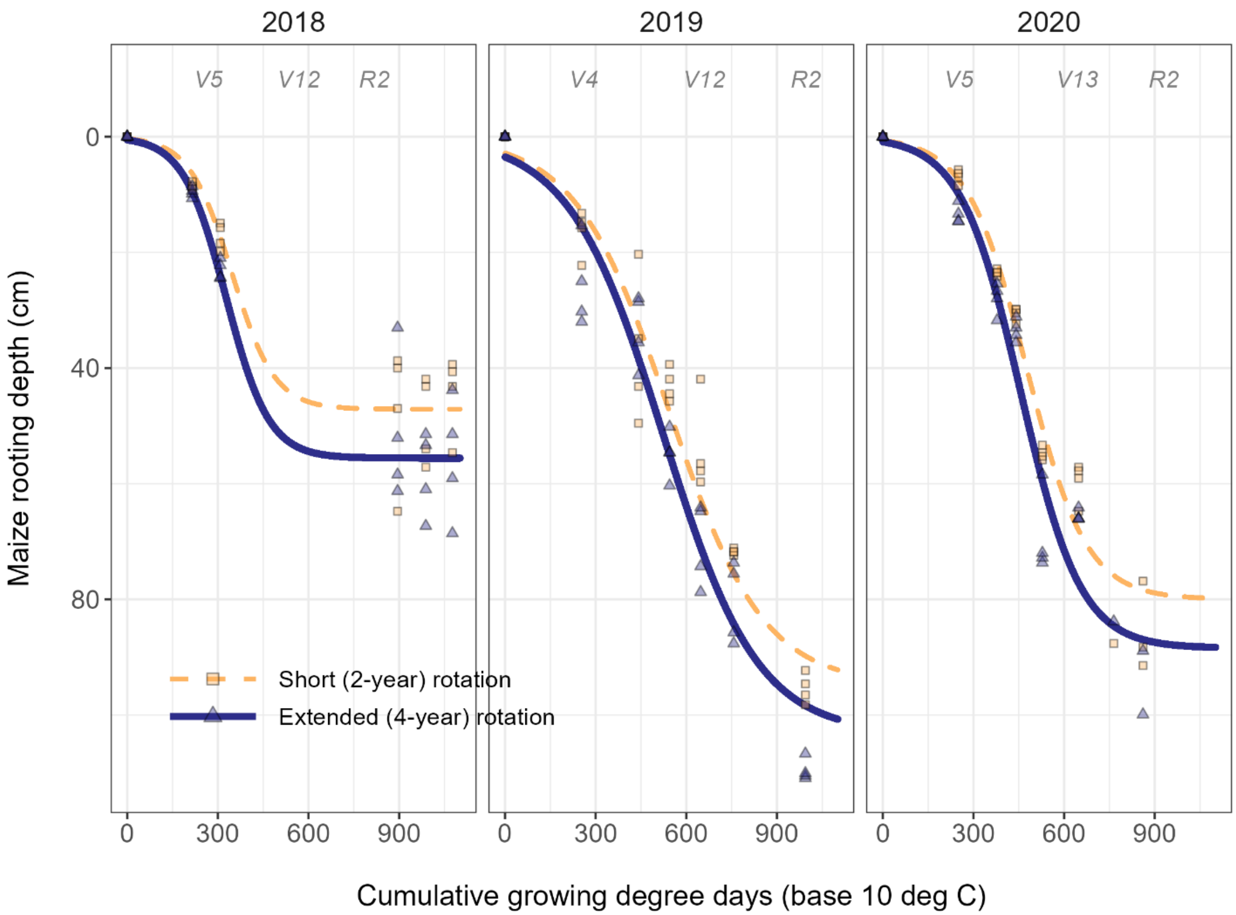

*Figure 2. Maize rooting depth across three growing seasons (2018, 2019, 2020; refer to Figure 1 for weather conditions) in short (yellow, dashed, squares) and extended (dark blue, solid, triangles) rotations; points represent individual measurements, lines represent the non-linear model fits, italic 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 greater 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) quantified 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**).

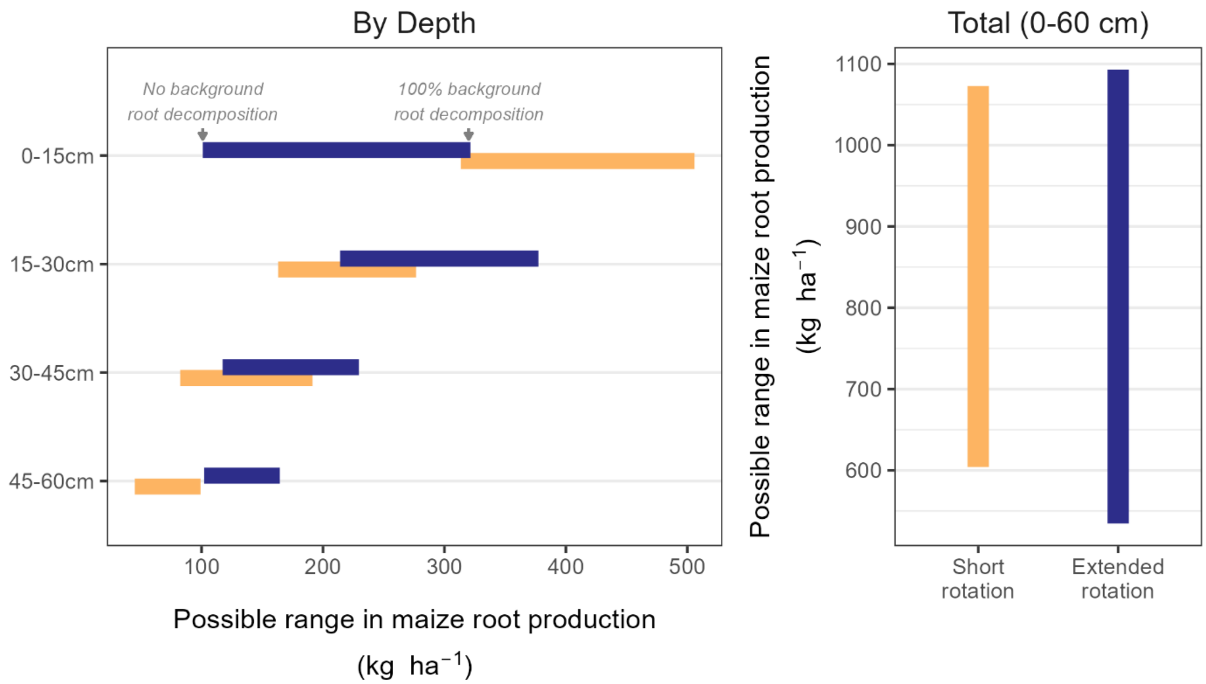

Figure 3. (Left) Possible ranges in mean maize root mass production (n=8) depending on assumed rate of decomposition of root mass present at planting ('background' roots) for maize grown in the short (yellow) and extended (dark blue) rotations separated by depth and (right) range in total possible root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

3.4 Growth analysis

In the years with a significant yield differential between the two rotations, the maximum aboveground maize biomass (*Asym*; Eqn. 1) in the extended rotation was significantly greater than in the short. However, in years where the two rotations' yields were not significantly different, the two rotation's maximum aboveground biomass was likewise not significantly different (**Table 3; Figure S2; Table S1**). In 2013 (a year with significant yield differences), the date at which maize achieved half of its maximum biomass (*xmid;* Eqn. 1) was significantly earlier in the extended rotation ($p = 0.05$) 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, in 2018 (another year with significant yield differences), *xmid* for the extended rotation occurred significantly *later* than for the short rotation ($p < 0.01$) 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 (2014, 2019, 2020). 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 rotation maize grain yield[†]	Extended rotation's maize maximum rooting depth/surface roots relative to short rotation	Timing of extended rotation's maize growth advantage[‡]	Ratio of extended:short rotation harvest index	Ratio of extended:short 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**). 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 of the extended rotation soil. 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°C, but soil temperature and moisture 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**). 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,' positioning the plant to access resources (i.e., water and nutrients) that may be, or may become, available in deeper soil layers (van der Bom et al. 2020). 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 hypothesis that '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.

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 (Han et al. 2015). 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, 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**).

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 is consistent with 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). Regardless, the patterns in penetration resistance are not consistent with the differences in roots observed in the present study.

While the rotation benefit observed in the present study was realized in a range of weather conditions (**Figure 1**), the rotation benefit 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 and a conceptual figure of our hypothesis

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

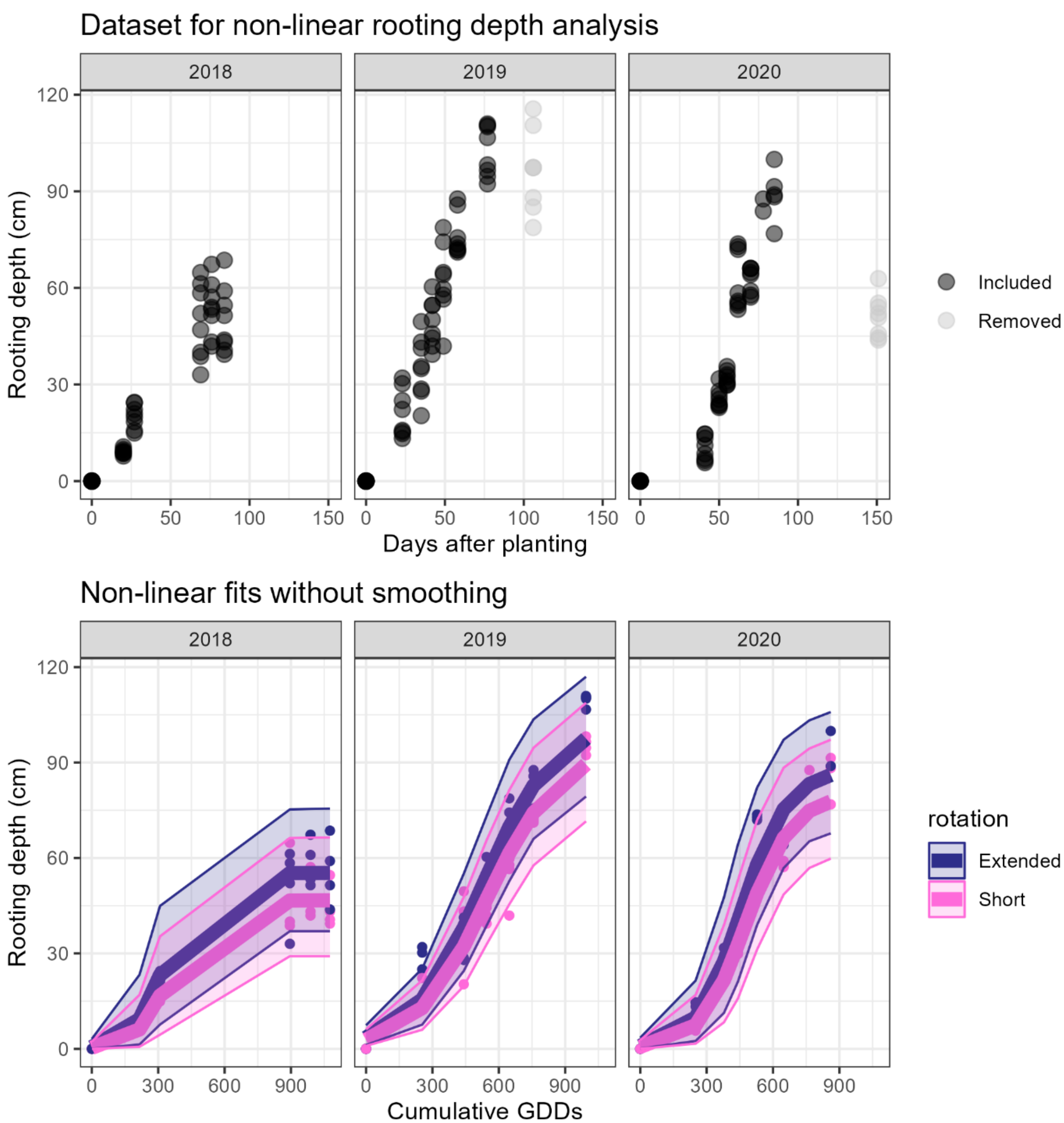


***Figure S1** – Maximum rooting depth over time, raw dataset and unsmoothed fitted values.*

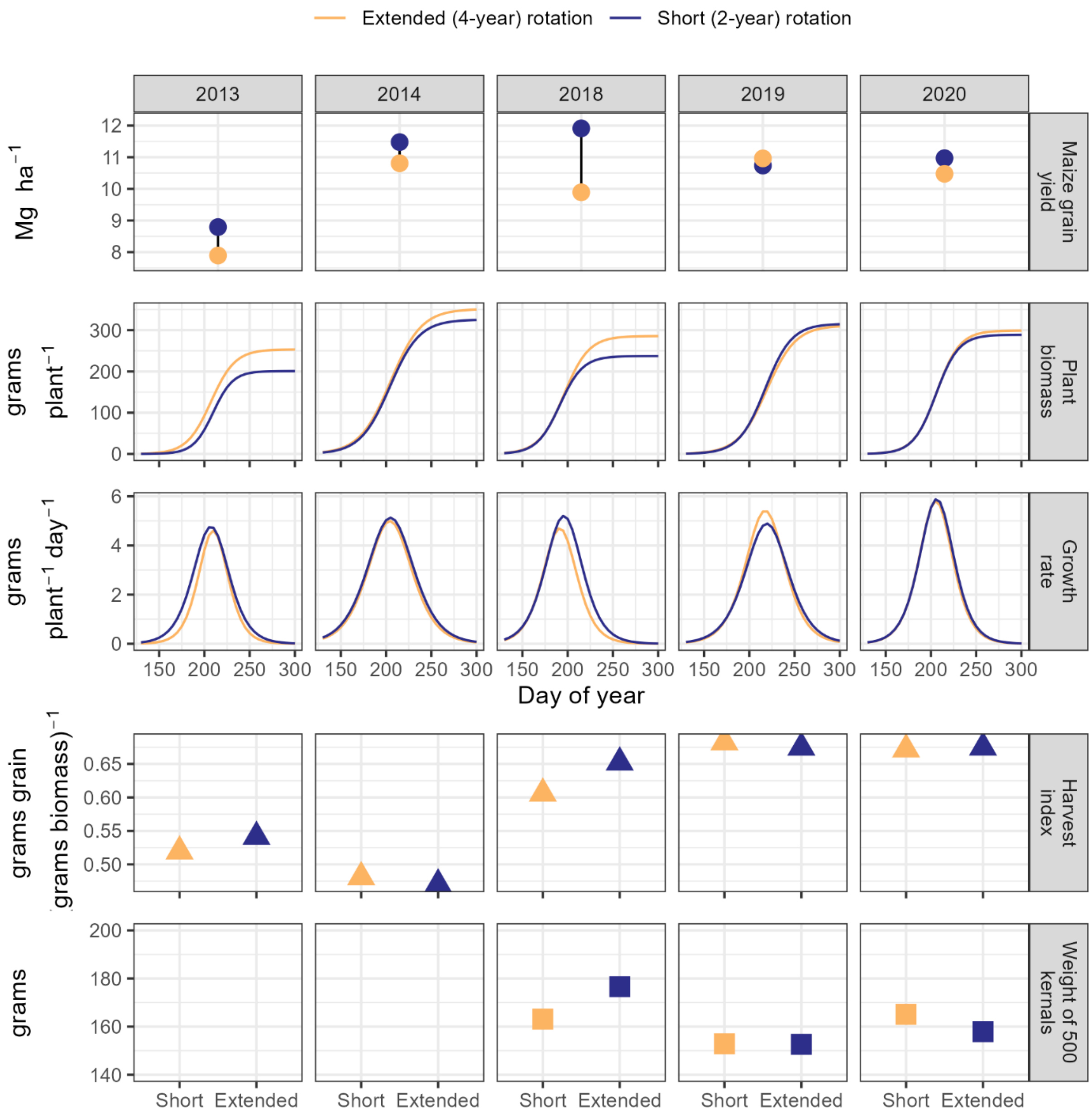


Figure S2. *Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight for the extended 4-year rotation (dark blue) and short 2-year rotation (yellow). The reader is directed to the manuscript text for indications of significant differences.*

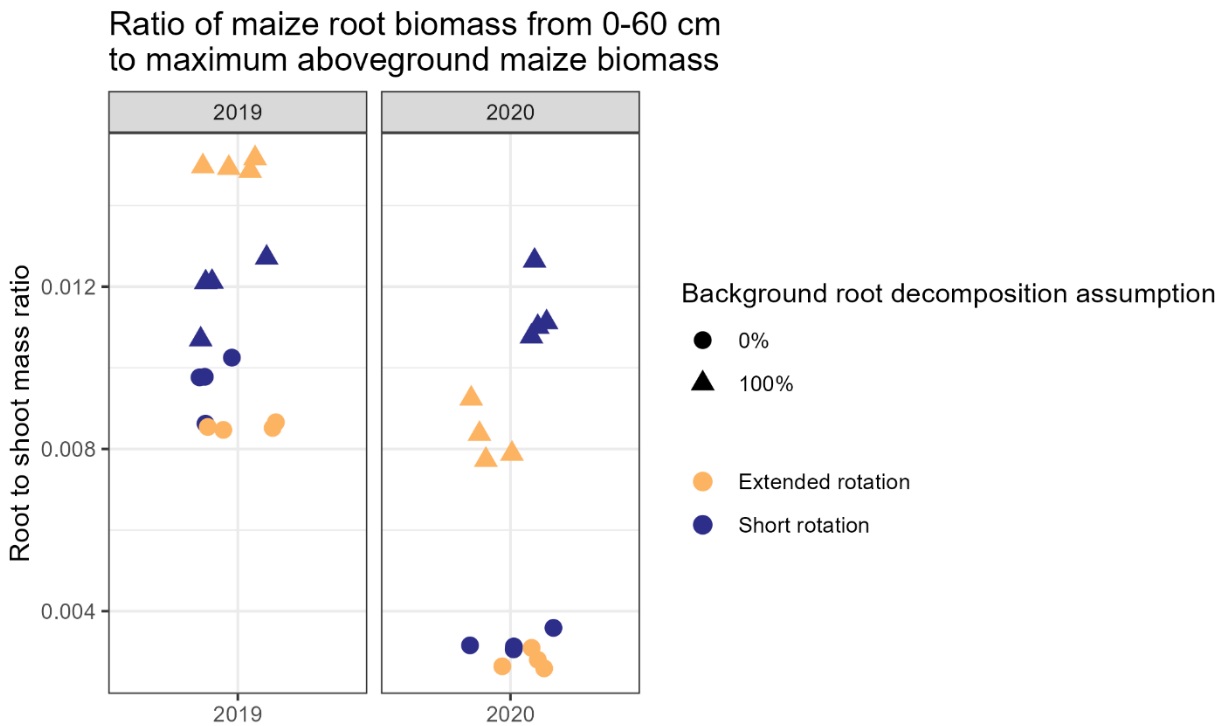


Figure S3. Root to shoot ratios for 2019 and 2020, with different background root decomposition assumptions.

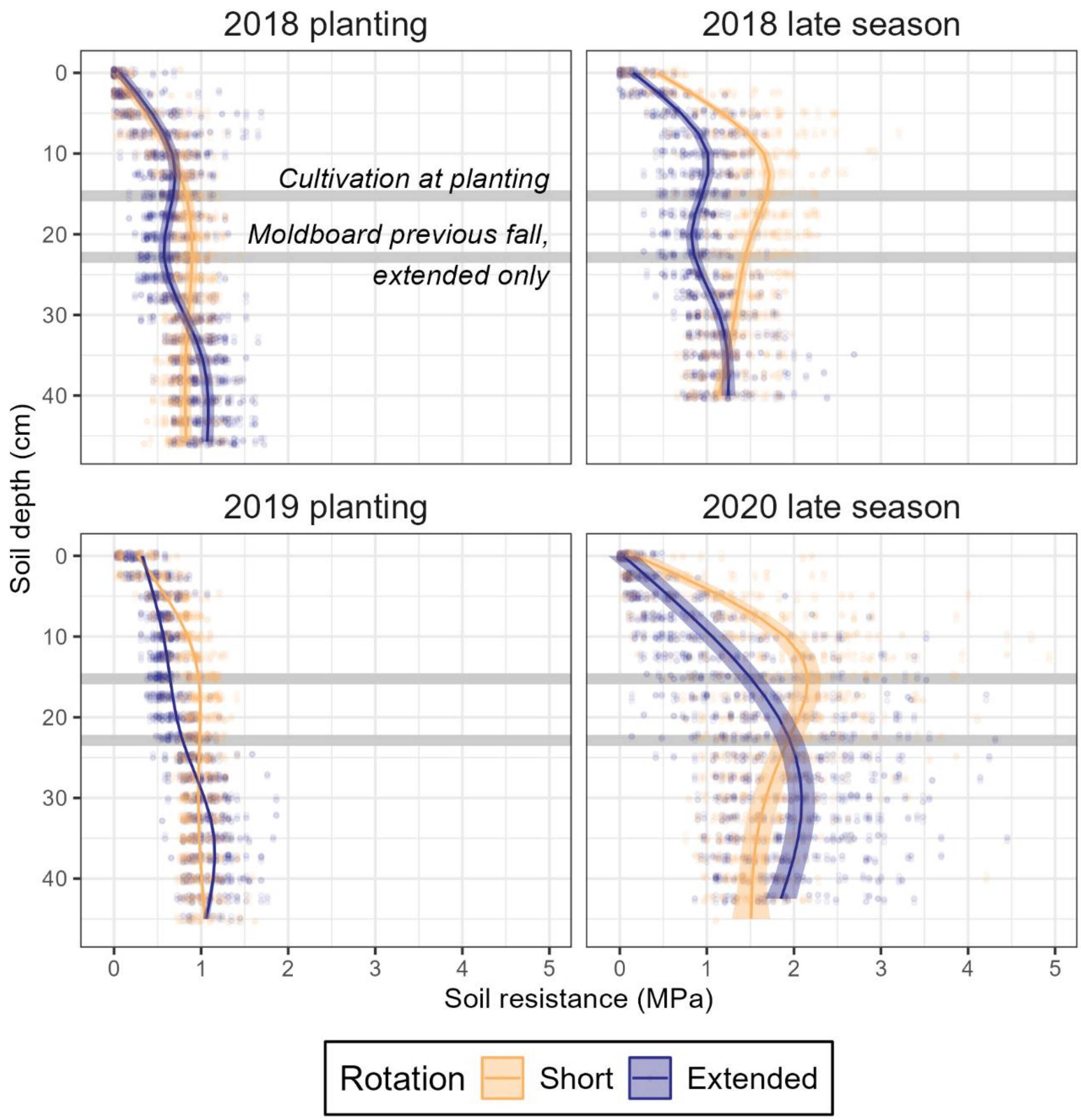


Figure S4. Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference.

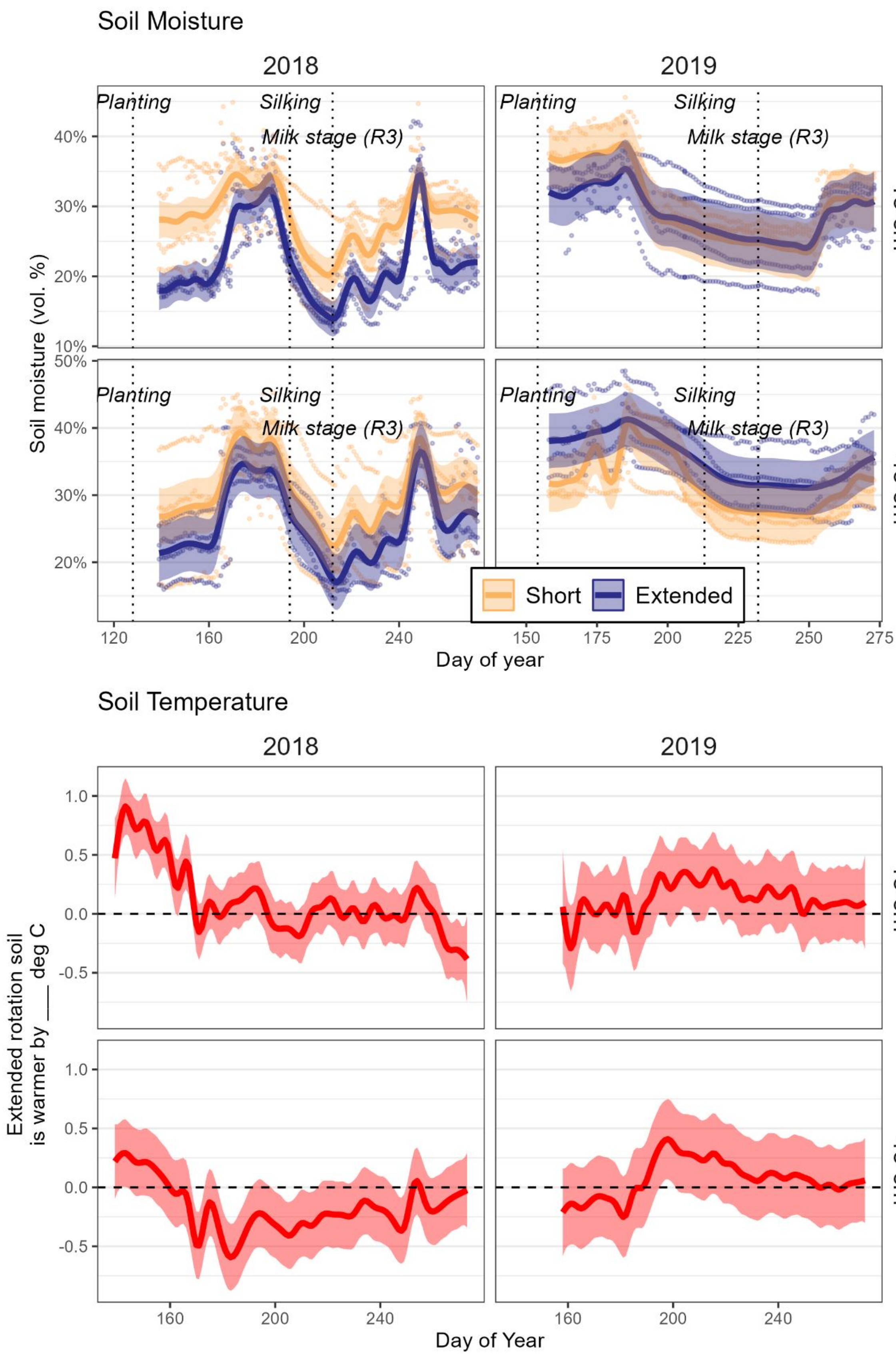

Soil Moisture
2018
2019
Planting
Silking
Milk stage (R3)
15 cm
45 cm
Soil moisture (vol. %)
Short
Extended
Day of year
Soil Temperature
Extended rotation soil is warmer by ___ deg C
Day of Year

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 (yellow) 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.

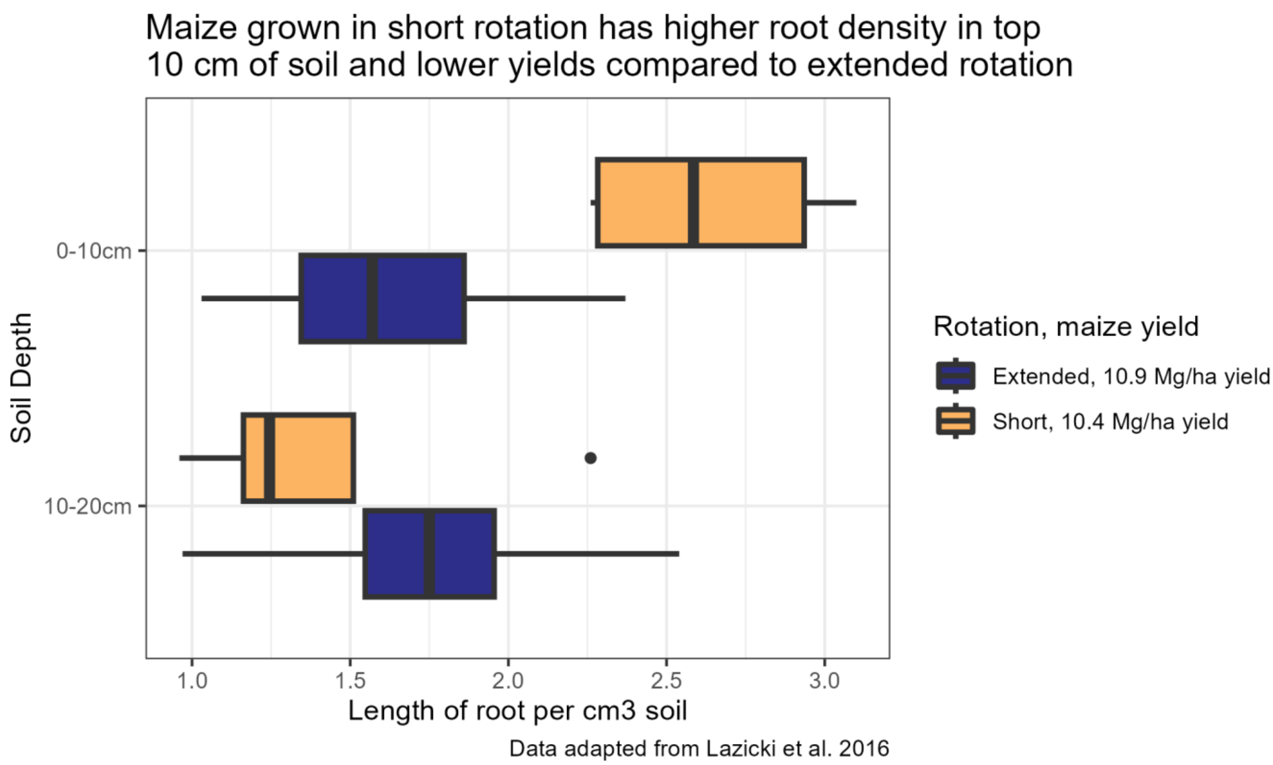
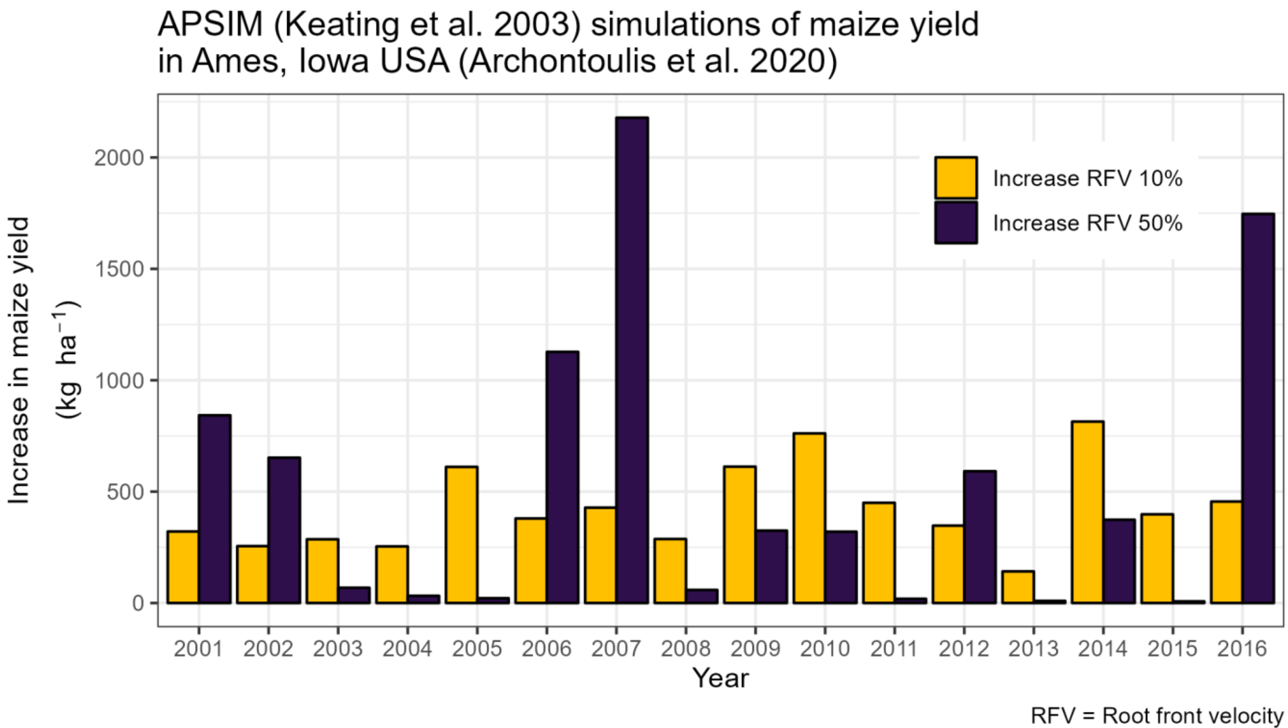


Figure S6 *– Data from Lazicki et al. 2016 shows differences in maize root structures coinciding with maize yield differences in short and extended maize rotations in 2009.*


APSIM (Keating et al. 2003) simulations of maize yield
in Ames, Iowa USA (Archontoulis et al. 2020)
Increase RFV 10%
Increase RFV 50%
Increase in maize yield
(kg ha^{-1})
2000
1500
1000
500
0
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Year
RFV = Root front velocity

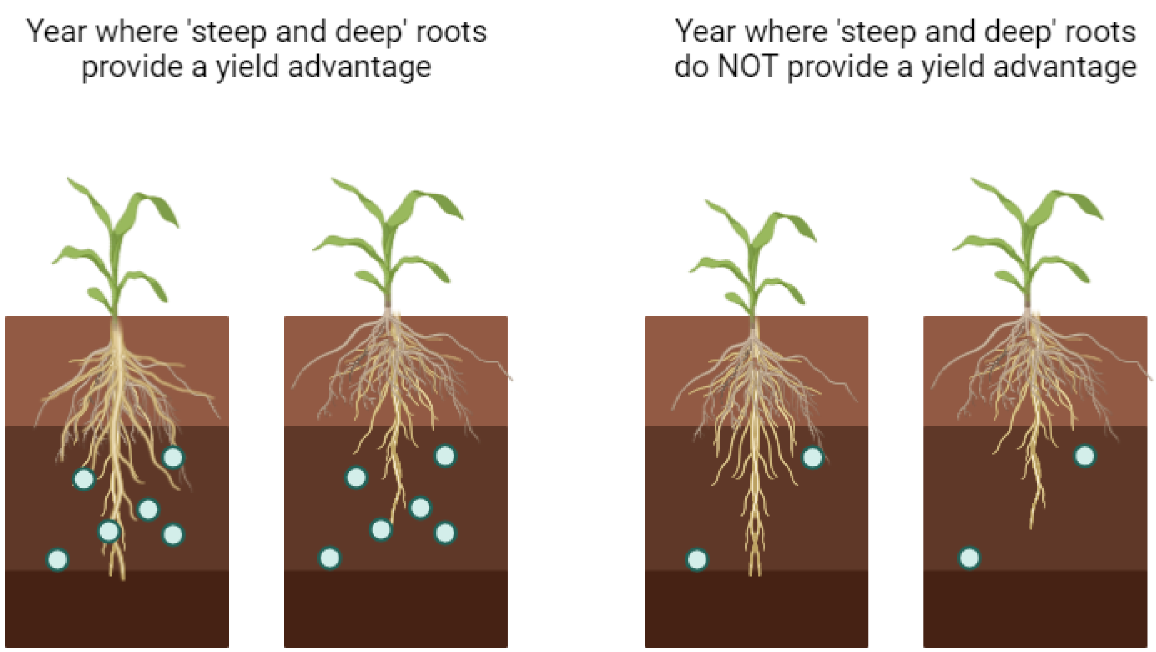

Year where 'steep and deep' roots
provide a yield advantage
Year where 'steep and deep' roots
do NOT provide a yield advantage

Figure S7 – (Top) *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.* ***(Bottom)*** *Conceptual figure.*

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

Note: **DF = Degrees of freedom*