

Field Crops Research

The roots of the rotation effect run deep

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Abstract:	<p>Context or problem It is well-established that maize (<i>Zea mays</i> L.) grown in complex cropping systems can require lower external nitrogen inputs to produce equal or higher grain yields compared to maize grown in simple systems. Understanding the mechanisms driving this phenomenon, commonly referred to as 'the rotation effect', is crucial for designing cropping systems that use land and resources efficiently. Differences in root systems can influence crop nutrient uptake and yield, but it is unknown if such differences play a role in the rotation effect.</p> <p>Research question We hypothesized that maize grown in a more complex rotation system invests less resources into root growth while achieving the same or higher above-ground biomass.</p> <p>Methods Using a long-term experiment in place since 2001, we measured root biomass in 15 cm increments from 0-60 cm, maximum rooting depth, and grain yields in the maize phase of two contrasting rotations: a simple 2-year rotation of maize-soybean (<i>Glycine max</i> [L.] Merr), and an extended 4-year rotation of maize-soybean-oat (<i>Avena sativa</i> L.)/alfalfa (<i>Medicago sativa</i> L.)-alfalfa that periodically received composted cattle manure. Additionally, we measured soil penetration resistance and soil moisture, and performed a growth analysis on aboveground maize biomass.</p> <p>Results From 2013-2020, maize grain yields averaged 8% higher in the complex compared to the simple rotation (11.0 and 10.2 dry Mg ha⁻¹, respectively). The timing (e.g., early season, late season) of the complex rotation's maize growth advantage over the simple rotation was not consistent across years. The maximum rooting depth of maize in the complex rotation was consistently deeper, by an average of 11% (82 versus 76 cm, respectively). From planting to physiological maturity, maize grown in the simple rotation added 34% more root mass in the 0-60 cm soil profile compared to the complex rotation. The simple system's maize had significantly more root biomass in the soil surface layer (0-15 cm), but below that depth the two systems' root mass did not differ. Soil penetration resistances from 0-30 cm were 30% higher in the simple versus complex rotation across the growing season, which may have partially contributed to the different root distributions.</p> <p>Conclusions We posit that the investment in roots deeper in the soil profile seen in the complex system does not guarantee higher maize yields, but instead increases the likelihood the plant can withstand unfavorable growing conditions and thus produce higher grain yields compared to maize grown in the simpler rotation.</p> <p>Implications To our knowledge, this is the first report of cropping system complexity impacting maize rooting depth. This study enhances our understanding of the 'rotation effect', and provides new data on yields and roots, both of which are highly relevant in this new era of soil carbon focus. Similar measurements taken in other locations would aid</p>

	in understanding the role of maize rooting depth and structure in the 'rotation effect' in additional contexts.
Suggested Reviewers:	<p>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.</p>
	<p>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.</p>
	<p>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.</p>

December 22, 2022

To the Editorial Board of Field Crops Research:

On behalf of all co-authors, herewith I am submitting a manuscript entitled “The roots of the rotation effect run deep” to be considered for publication in Field Crops Research. This manuscript contains new information on how cropping systems impact crop root characteristics. We performed this study to address a lack of data concerning the link between ‘the rotation effect’, wherein crop yields increase as the crop is grown in longer rotations, and crop roots. Our study offers several innovative aspects:

- Comprehensive study of maize grown in different cropping systems that includes above-ground plant, below-ground root, and soil information across the growing season
- Estimates of maize rooting depth across the growing season in contrasting cropping systems
- Quantification of maize root additions across the growing season in contrasting cropping systems

This unique dataset allowed us to address a large knowledge gap concerning how crop roots respond to different cropping system histories, and how those responses are associated with crop yields. Our analyses indicate differences in crop roots may be a key component in explaining ‘the rotation effect’. Given the relevance of this finding to field crops, we believe this contribution is a very relevant to your journal.

Sincerely,

A handwritten signature in black ink, appearing to read 'Virginia (Gina) Nichols', with a stylized, flowing script.

Virginia (Gina) Nichols
Post-doctoral scholar
Department of Plant Sciences
University of California, Davis

The roots of the rotation effect run deep

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Highlights:

- Over eight years, maize grown in a complex four-year rotation yielded an average of 8% higher than maize grown in a simple two-year rotation
- The maximum rooting depth of maize grown in a complex rotation was 11% deeper compared to maize grown in a simple rotation
- Maize grown in the complex rotation had 34% less root mass in the 0-60 cm soil depth, with reductions occurring mainly in the 0-15 cm soil profile
- Maize grown in a complex rotation had a ‘deeper and cheaper’ root structure compared to maize grown in a simple rotation

Abstract

Context or problem

It is well-established that maize (*Zea mays* L.) grown in complex cropping systems can require lower external nitrogen inputs to produce equal or higher grain yields compared to maize grown in simple systems. Understanding the mechanisms driving this phenomenon, commonly referred

to as ‘the rotation effect’, is crucial for designing cropping systems that use land and resources efficiently. Differences in root systems can influence crop nutrient uptake and yield, but it is unknown if such differences play a role in the rotation effect.

Research question

We hypothesized that maize grown in a more complex rotation system invests less resources into root growth while achieving the same or higher above-ground biomass.

Methods

Using a long-term experiment in place since 2001, we measured root biomass in 15 cm increments from 0-60 cm, maximum rooting depth, and grain yields in the maize phase of two contrasting rotations: a simple 2-year rotation of maize-soybean (*Glycine max* [L.] Merr), and an extended 4-year rotation of maize-soybean-oat (*Avena sativa* L.)/alfalfa (*Medicago sativa* L.)-alfalfa that periodically received composted cattle manure. 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 averaged 8% higher in the complex compared to the simple rotation (11.0 and 10.2 dry Mg ha⁻¹, respectively). The timing (e.g., early season, late season) of the complex rotation’s maize growth advantage over the simple rotation was not consistent across years. The maximum rooting depth of maize in the complex rotation was consistently deeper, by an average of 11% (82 versus 76 cm, respectively). From planting to physiological maturity, maize grown in the simple rotation added 34% more root mass in the 0-60 cm soil profile compared to the complex rotation. The simple system’s maize had significantly more root biomass in the soil surface layer (0-15 cm), but below that depth the two systems’ root mass did

not differ. Soil penetration resistances from 0-30 cm were 30% higher in the simple versus complex rotation across the growing season, which may have partially contributed to the different root distributions.

Conclusions

We posit that the investment in roots deeper in the soil profile seen in the complex system does not guarantee higher maize yields, but instead increases the likelihood the plant can withstand unfavorable growing conditions and thus produce higher grain yields compared to maize grown in the simpler rotation.

Implications

To our knowledge, this is the first report of cropping system complexity impacting maize rooting depth. This study enhances our understanding of the ‘rotation effect’, and provides new data on yields and roots, both of which are highly relevant in this new era of soil carbon focus. Similar measurements taken in other locations would aid in understanding the role of maize rooting depth and structure in the ‘rotation effect’ in additional contexts.

Keywords

Maize; crop rotation, roots, penetration resistance; alfalfa

1 Introduction

Over the past 60 years, the diversity of maize (*Zea mays* L.) production systems in the Midwestern United States (US) has been reduced from multi-species rotations that included small grains and forage legumes to maize monocultures or simple alternations of maize and soybean (*Glycine max* [L.] Merr) (Aguilar et al., 2015; Hijmans et al., 2016; Crossley et al., 2021). Presently, five states in the Midwestern US produce approximately one-sixth of the world’s maize and soybean grain (FAO, 2020; USDA National Agricultural Statistics Service

2021), and it follows that in the Midwestern US a significant amount of agricultural land is dedicated to a simplified maize/soybean system (Boryan et al., 2011; USDA National Agricultural Statistics Service Cropland Data Layer, 2021). Several unintended, but nonetheless undesirable outcomes have accompanied this simplification including but not limited to increased rates of soil erosion, increased risk of flooding, and increased risks of nitrate pollution (Hatfield et al., 2009, 2013; Schilling et al., 2010; Jones et al., 2018; Pasley et al., 2021). While there are numerous barriers to re-diversifying Midwestern systems (Mortensen and Smith, 2020; Weisberger et al., 2021), there is value in understanding the mechanisms that may enable crop diversification to mitigate these negative outcomes.

Midwestern maize-based systems fall into three main categories: (1) continuous maize systems, wherein maize is grown for two or more consecutive seasons; (2) simple rotated maize systems, wherein maize is rotated with soybean; and (3) extended maize rotations, wherein maize is grown in a rotation with two or more years between maize crops, often including a small grain and/or a forage crop. As maize cropping systems move from monocultures to simple rotations or from simple 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). The former phenomenon is commonly referred to as the ‘continuous maize penalty’, and the latter ‘the rotation effect’. To our knowledge it is unclear whether they are distinct expressions of unique mechanisms, or if they represent a continuum of the same mechanistic underpinnings.

There have been numerous studies in the U.S. 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 simple 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 driving mechanisms behind the rotation effect remain uncertain. In a long-term cropping systems research experiment in Iowa (Liebman et al., 2008; Davis et al., 2012) researchers have found differences in the vertical distributions of resources, microbial communities, and nutrient cycling activity in soil profiles of simple and complex maize systems (Lazicki et al., 2016; King and Hofmockel, 2017; Osterholz et al., 2018; Poffenbarger et al., 2020; Baldwin-Kordick et al., 2022). However, it is unclear how these altered distributions are linked to higher maize yields in the complex rotations.

Both biological and physical differences in soils can impact crop root structures. Diverse rotations may alter soil physical structure, which may in turn promote different crop rooting structures (Ball et al. 2005). An analysis of maize root- and soil-associated bacterial and fungal communities showed differences between simple versus complex rotation systems, with a greenhouse study suggesting these soil biological differences were associated with different root architectures (Bay et al. 2021). Lazicki et al. (2016) found differences in maize root distributions in plots with varying cropping system histories, but the data were limited to shallow depths and did not control for previous crop root carryover, which can impact interpretations (Hirte et al. 2017). It is feasible that, like the continuous maize penalty, differences in maize root structures

contribute to increased resource capture in complex rotations, thus driving higher maize yields compared to simpler systems.

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). We hypothesized that maize grown in complex rotations develops a root structure that is better able to capture resources with less investment compared to the simple rotation. To test this hypothesis, we made the following measurements in the maize phase of simple (maize-soybean) and complex (maize-soybean-oats/alfalfa-alfalfa) rotations:

1. Maize grain yields (2013-2020)
2. Maize root biomass as a proxy for the resources invested by the maize crop into roots (2019-2020)
3. Maximum maize rooting depth as a proxy for the soil space made available for resource capture by that investment (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 (2018-2020); and soil moisture measurements (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 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 85 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 simple rotation), and a 4-year rotation of maize-soybean-oat/alfalfa-alfalfa that periodically received composted cattle manure (hereafter the complex rotation). 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 sampled in this study; only the maize phase (bolded) was sampled in this study; for more details see Liebman et al. 2008 and Hunt et al. 2020

Rotation	Crop sequence	Nitrogen sources	Tillage regime
Simple 2-year	Soybean- Maize	Mean total of 180 kg ha ⁻¹ inorganic nitrogen applied to maize phase, with 112 kg ha ⁻¹ applied at planting and the remaining at V6 side-dressing based on soil nitrate sampling	Fall chisel plowing and spring field cultivation preceding soybean planting, spring surface cultivation preceding maize planting
Complex 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	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 15.0 (SAS Institute, Cary, North Carolina, USA). Additional R packages were used for specific analyses as described below. Significance for statistical tests was set at $\alpha=0.05$ 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. The R code

used in these analyses is available in a Github repository (will be made public once manuscript is accepted for publication).

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

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

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

2.5 Rooting depth

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

Eqn. 1

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

193 where $rootdepth(GDD)$ is the maximum rooting depth at a given cumulative GDD after planting
194 and A_{sym} , x_{mid} , and $scal$ are estimated parameters. A_{sym} represents the maximum rooting depth
195 achieved, x_{mid} represents the cumulative GDD value at which half of the maximum rooting depth
196 was achieved, and $scal$ describes the steepness of the curve (Miguez et al., 2018). We found the
197 best model fit incorporated a fixed effect of rotation on A_{sym} , $scal$, and x_{mid} ; a random effect of a
198 year-factor on A_{sym} , x_{mid} , and $scal$; a random effect of block on A_{sym} ; and a power variance
199 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 the middle rows of the plots, approximately 4.6 m x 84 m. All yields are reported on a dry weight basis.
Maize above-ground biomass	2013, 2014, 2018, 2019, 2020	Maize above-ground biomass was measured approximately every two weeks throughout the season. Eight plants were cut at ground level, separated into leaf, stem, and reproductive components, dried at 60° C for at least 72 hours, then weighed.
Maize maximum rooting depth	2018, 2019, 2020	Maximum maize rooting depth was measured starting at 20, 23, and 41 days after planting in 2018, 2019, and 2020, respectively. Sampling was repeated approximately every 2 weeks until 84, 106, and 151 days after planting in 2018, 2019, and 2020, respectively. Maximum rooting depth was determined visually using the protocol of Ordóñez et al. (2018). A soil core was drawn with a 19-mm-diameter soil probe, white roots were identified, and their depth estimated to the nearest cm using a meter stick. Samples were repeatedly taken 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 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).

2.6 Root mass

Distinguishing newly added root biomass from root biomass from the previous year's crop and weed roots is difficult using visual sorting, and failure to address these background levels of roots can lead to overestimates of root mass with the overestimate varying by cropping history (Hirte et al. 2017). To address this issue, the three main options are 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 (see **Table S1**), and therefore report the production of new crop root biomass as a relative value: the difference between the estimated root biomass at peak crop biomass and the measured root biomass shortly after planting. If there was less root material present at the peak biomass sampling than at the baseline, the relative root biomass would therefore be negative. Additional details of sampling protocols are presented in **Table 2**. Maize root production was estimated as the change in root mass from the start of each growing season (3 days after planting (DAP) in 2019 and 4 DAP in 2020; **Table 2**) and the last sampling date. Thus, the amount of root mass retained in the soil of each plot from the previous crops was used as a baseline for assessing subsequent changes in root mass during the maize growing season.

The Restricted Maximum Likelihood (REML) method for linear mixed effect models in JMP Pro 15.0 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). 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.

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 for ease in interpretation. A separate model was fit for each rotation in each year to allow for derivatives to be taken for each rotation in each year. The first- and second-order derivatives of the fitted equation were used as estimates of the absolute and relative growth rates, respectively. 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 net 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, 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 values, rather than absolute.

2.9 Penetration resistance

Penetration resistance was statistically modelled separately for each year and date of sampling using a generalized additive mixed model with a fixed intercept effect of rotation treatment, a fixed ‘wiggle’ component of rotation treatment, five knots, and a random ‘wiggle’ effect of block. Generalized additive models can model highly non-linear relationships and are

useful when the goal is to compare treatments rather than to create predictions. The *gamm* function of the R package *mgcv* (McCulloch and Neuhaus, 2005; Wood, 2011) was used, and the *emmeans* package was used to assess pairwise comparison significance. Models were fit using both the raw and square-root-transformed data. Although the model on the transformed data produced a better fit according to inspection of residuals, statistical conclusions were not different in the two models so the results from the untransformed data are presented for ease in interpretation.

2.10 Soil moisture

The hourly soil moisture data were averaged over a 24-hour period (12:00 am to 11:59 pm) for analysis. The daily means were statistically modelled 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 complex 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 simple rotation yielded a mean of 10.2 dry Mg ha⁻¹, while maize grown in the complex rotation yielded 8% higher with a mean grain yield of 11.0 dry Mg ha⁻¹.

Over the past 30 years, from 15-April through 15-October the research site averaged 597 mm of precipitation with a mean air temperature of 19° C. From 2013-2020, growing season

weather conditions varied considerably compared to these long-term averages, with the grain yield dataset capturing conditions in all four temperature/precipitation combinations (**Figure 1**). Years that included growth analyses were likewise represented in all four quadrants, and the three years with full datasets (grain yields, growth analysis, root data) represented all but cool and wet conditions.

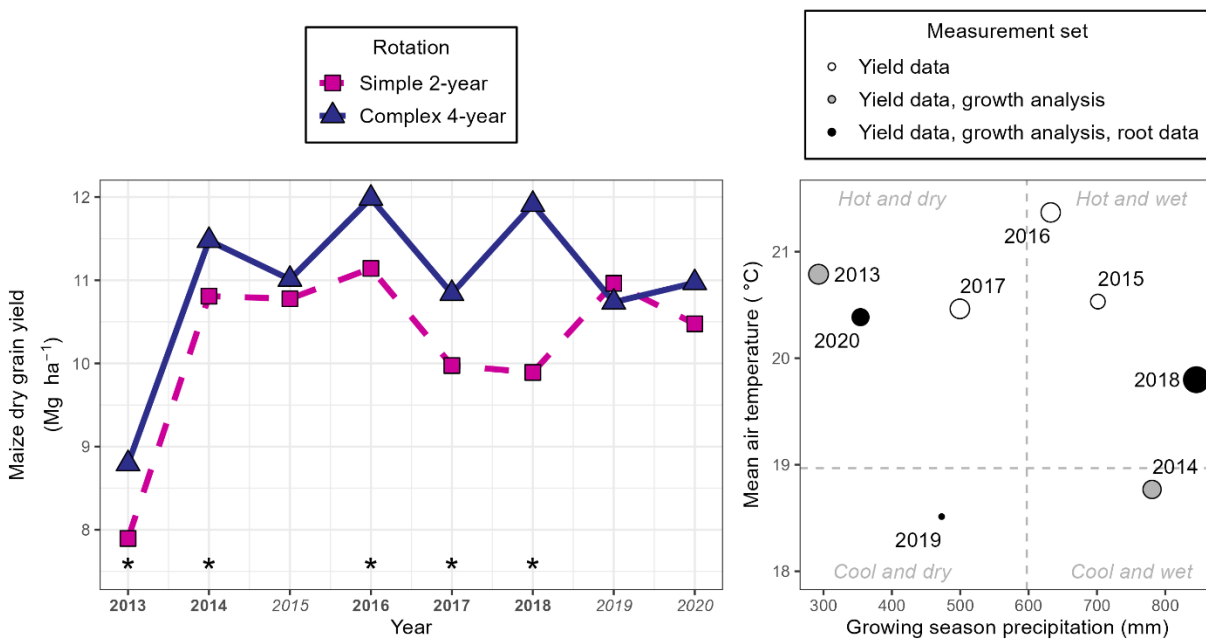


Figure 1. Maize yields and growing season weather; (left) mean maize grain yields ($n = 4$) in the complex (dark blue, solid, triangles) and simple (pink, dashed, squares) rotations from 2013-2020 (lines connecting points for ease in viewing) and significantly different yields ($p < 0.01$) indicated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

3.2 Rooting depth and root mass

The rooting depth of maize grown in the complex rotation trended consistently deeper in the majority of sampling times in all three growing seasons (**Figure 2**). Rotation affected maize maximum rooting depth (A_{sym} ; $p < 0.01$), estimated at 11% deeper in the complex rotation compared to the simple rotation (82 cm and 76 cm, respectively). While the complex rotation roots also descended faster, the effect was not statistically significant (x_{mid} ; $p = 0.19$; **Table S4**).

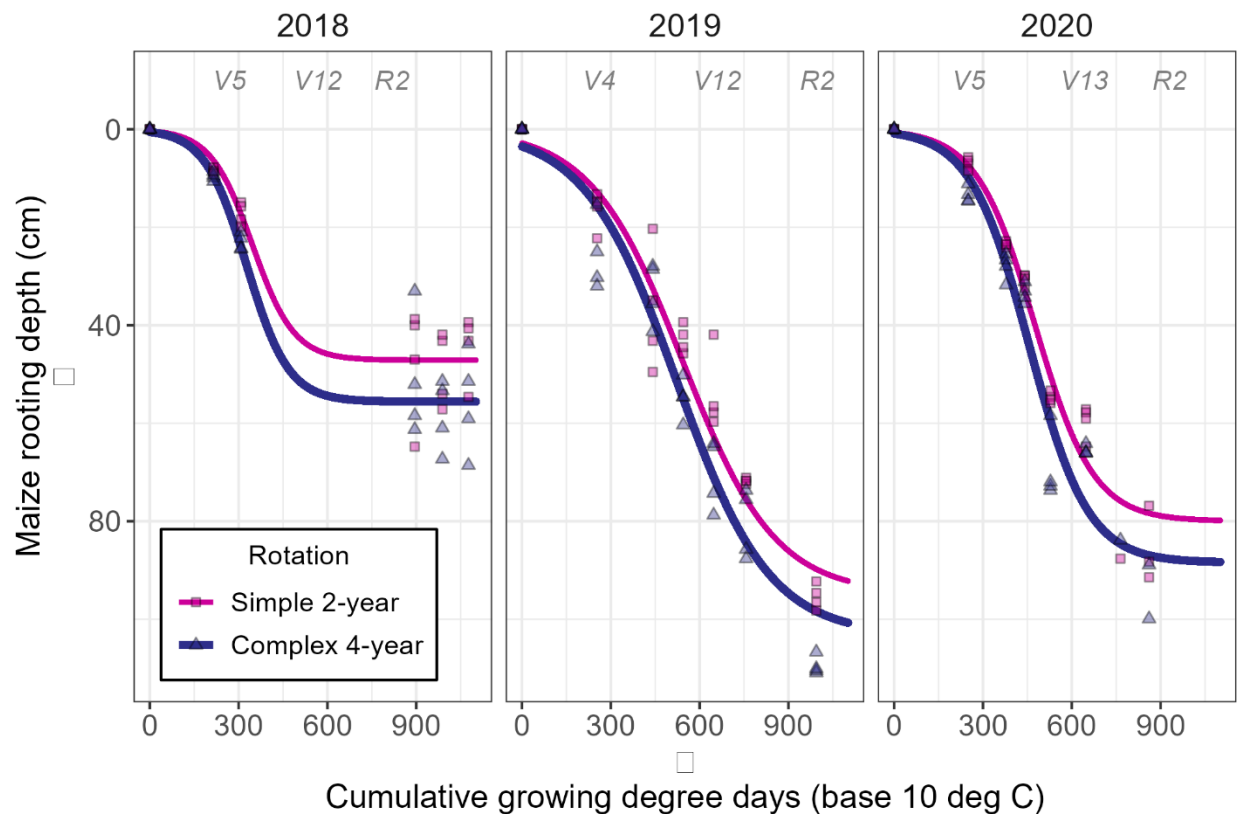
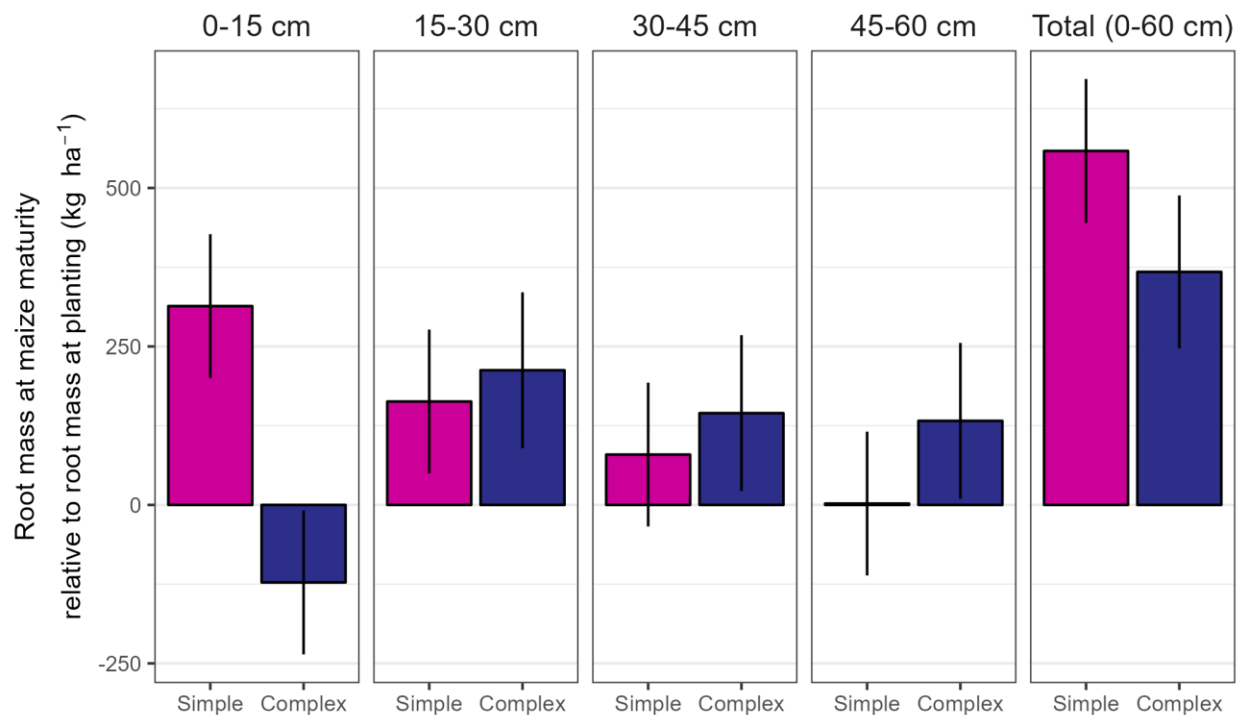


Figure 2. Maize rooting depth across three growing seasons (2018, 2019, 2020; refer to Figure 1 for weather conditions) in simple (2-year, pink) and complex (4-year, dark blue) rotations; points represent individual measurements, lines represent the non-linear model fits, italic text is the maize stage; model uncertainty is omitted for simplicity but is available in supplemental material; first points at 0 depth indicate maize planting date.

93 The effect of rotation treatment on root mass differed significantly by sampling depth
 94 ($p=0.01$). Year and its interactions did not have significant effects (**Table S2**), so results are
 95 presented as marginal estimates over years (**Figure 3**). At the 0-15 cm depth increment, the root
 96 mass added in the two systems differed significantly ($p = 0.02$), with the simple system
 97 increasing by 314 kg ha^{-1} (SE:113) from the baseline measurements, while the complex
 98 rotation's root mass decreased by 122 kg ha^{-1} (SE:113) from the baseline measurement. At all
 99 other depths, the difference between the complex and simple system's additions was not
 100 statistically significant (**Table S3**).



101
 102 *Figure 3. Root mass at maize maturity relative to root mass present at planting at each soil depth*
 103 *for maize grown in the simple (2-year rotation, pink) and complex (4-year rotation, dark blue)*
 104 *systems, bars represent marginal means and vertical lines the standard error of the mean.*

3.3 Growth analysis

The maximum aboveground maize biomass ($Asym$; Eqn. 1) as estimated from the growth analysis was significantly higher in the complex rotation in the years when the complex rotation yielded significantly higher grain yields (**Table 3; Figure S3; Table S4**). The date at which the maize achieved half of its maximum biomass ($xmid$; Eqn. 1) was significantly earlier in the complex system in 2013 ($p = 0.05$), significantly later in 2018 ($p < 0.01$), and was not significantly different in any other year. The maximum growth rates in the two systems ($scal$; Eqn. 1) were not significantly different in any year. 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 without a strong rotation effect.

The mean root-to-shoot ratios tended to be higher in the simple rotation compared to the complex rotation (**Figure S4**), averaging 0.0095 compared to 0.0085, but the differences were not statistically significant. 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 differed. The present study was focused on assessing the impact of cropping system history on maize roots, and as such the maize crop was grown following different crops (soybeans, alfalfa). We therefore had to control for the differences in root legacies left by the previous crop (see Section 2.6), while in other studies the previous crop is constant. Because our study used a background sample taken at planting as a baseline, it is expected that using the net root additions would result in a lower estimate of root biomass compared to raw measurements taken at maize physiological maturity. Another study using in-growth root cores found maize added 480-560 kg ha⁻¹ in root material over the growing season in the top 30 cm of soil (Dietzel et al. 2017). These values are comparable to those found in our study (400-670 kg ha⁻¹, Figure 3).

3.4 Penetration resistance

Penetration resistance above 30 cm soil depth was consistently lower in the complex rotation, regardless of year or sampling period (planting, late season; **Figure S5**). From 0-30 cm, the simple and complex rotations had mean penetration resistances of 0.7 and 0.6 MPa at planting, and 1.5 and 1.1 MPa at late season sampling, respectively. This corresponded to a 23 and 37% increase in resistance in the top 30 cm in the simple system at planting and late-season sampling, respectively. From 30 to 45 cm the complex system tended to have higher penetration resistance by an average of 15% regardless of year or sampling time (1.1 MPa/0.9 MPa at planting, and 1.7/1.4 MPa in the late season, respectively).

148 *Table 3. Summary of complex rotation's effects on grain yields, growth analysis, and yield*
149 *components for years with growth analysis data, ordered by magnitude of rotation effect*

Ratio of complex:simple system maize grain yield†	Timing of complex rotation's maize growth advantage	Ratio of complex:simple harvest index	Ratio of complex:simple 500-kernal weight	Year
1.20***	Late season***	1.08***	1.13**	2018
1.10**	Early season**	1.04**	-	2013
1.06*	ns	ns	-	2014
ns	ns	ns	ns	2020
ns	ns	ns	ns	2019

†Ordered by largest to smallest estimated rotation effect on maize grain yield

* p<0.10, **p<0.05, ***p<0.01, ns: not significant

- indicates no data was collected that year

150 **3.5 Soil moisture**

151 In both years of measurement, the complex system's soil moisture at 15 cm depth was
152 significantly lower for the first month following planting (**Figure S6**). After that period, the

effects were not consistent across years; in 2018 the simple system soil was consistently wetter than the complex system's soil, but in 2019 there was no difference. The same patterns were present at the 45 cm depth.

4 Discussion

In this study, we tested the hypothesis that maize grown in a more complex rotation system invests less resources into root growth but produces a root system that is associated with an increased likelihood of higher grain yields compared to maize in the simple rotation. While our data do not establish direct cause-effect relationships, they are consistent with this hypothesis and indicate that maize grown in the complex rotation invested less resources in shallow roots and achieved a deeper and more functional root structure compared to maize in the simple rotation. The timing of the maize growth advantage was not consistent across years (Table 3), suggesting the 'deeper and cheaper' root layout in the complex rotation provided resilience against unfavorable growing conditions, regardless of their timing, leading to increased resource acquisition and significantly higher grain yields in some years. We posit that the investment in roots deeper in the soil profile seen in the complex system does not guarantee higher maize yields, but rather it increases the likelihood the plant can withstand certain unfavorable growing conditions. Our study suggests this is a 'no-cost' benefit, as evidenced by the maize grown in the complex system achieving equal or higher grain yields compared to the maize grown in the simple system (**Figure 1**). While our dataset is not conclusive, it is consistent with our hypotheses and provides novel information that enriches our knowledge base on the rotation effect.

The maximum rooting depths in 2018 were shallow (~50 cm) due to an extremely wet year with consistently shallow water tables as documented at a nearby experimental site (Ebrahimi et al. 2019). Even under those conditions, the maize grown in the complex rotation had a deeper maximum rooting depth, and the yield differential between the complex and simple rotation maize was the highest recorded from 2013-2020 (Figure 1; Table 2). While the rotation benefit is realized in a range of weather conditions (Figure 1), it trends towards being maximized in ‘extreme’ precipitation years. Climate change scenarios project increased occurrences of precipitation extremes (Zhang et al. 2013), suggesting complex rotations may play an important role in building cropping system resilience to climate change. In 2019 and 2020, the maximum rooting depths were consistent with the ranges found by Ordóñez et al. (2018) across 10 site-years in Iowa, with the maize in the complex rotation again achieving a deeper maximum rooting depth.

The distribution of root mass in the two rotation systems differed, as did the total root mass added. The maize grown in the simple rotation added more root mass in the 0-15 cm depth compared to the maize in the complex rotation, but below 15 cm there was no difference in the added root mass (**Figure 3**). Previous work has shown the depth to the water table is associated with differences in maize root distributions (Nichols et al. 2019), but to our knowledge there are no reports of cropping system histories affecting maize root distributions. In the present study, we measured water table heights in two plots in 2019 (unpublished data) and found 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. In addition to adding less root mass to the shallow soil profile (0-15 cm), maize grown in the

complex rotation achieved a deeper maximum rooting depth in all three years of measurement, which covered widely ranging environmental conditions (**Figure 1**).

Across the entire 0-60 cm soil profile, the investment in total maize root mass was greater in the simple rotation than in the more complex rotation (**Figure 3**). There was no significant effect of year on the differences in root mass, suggesting the root differences may be consistent across years. While the lower investment in shallow roots and deeper rooting depths were consistent characteristics of maize grown in the more complex rotation, the part of the growing season in which the deeper roots are advantageous will differ from year to year, as evidenced by the inconsistency in the maize growth advantage timing, and the fact that the complex rotation did not have higher grain yields compared to the simple rotation in every year (Table 3). The root layout may provide the plant with greater nitrogen uptake (Osterholz et al. 2018), greater mobilization of resources to support grain growth resulting in a higher harvest index when those processes are limiting factors in the simple systems.

The causes for the deeper and cheaper root investments in the complex rotation cannot be discerned from this study alone, and might be physical, chemical, or biological. The root legacy of the crop preceding maize may impact the maize's root distribution. One study using mini-rhizotrons showed that when a maize crop followed an alfalfa crop, 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 maize, with estimated maximum rooting depths of 177 cm compared to 118 cm (Fan et al. 2016). Therefore, 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. Furthermore, decayed

alfalfa roots can increase soil water infiltration (Guo et al. 2019), which could lead to better drainage and contribute to the lower soil moisture levels seen in the complex rotation early in the season.

In addition to root legacies, changes in bulk density may also contribute to differences in root structures and investments. The complex rotation may have caused reduced soil bulk density due to 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). Bulk density differences were supported by the lower penetration resistance at planting above the moldboard plowing depths which were observed in the present as well as past studies (Baldwin-Kordick et al., 2022). While neither system had absolute resistances high enough to meaningfully impede root penetration, the resistances were measured in wet soils and therefore represent minimum values. The differences in penetration resistance (0.1-0.4 MPa) were of a magnitude that could 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).

In addition to the raw physical implications of lower bulk densities/resistances, there may be biophysical and chemical implications (Ball et al. 2005). Lower resistances could be indicative of better aeration, and possibly better water drainage or higher water uptake by the plants. Indeed, the soil water profiles showed drier soils after planting in the complex rotation compared to the simple rotation in both years of measurement. It is possible that the drier soils drove deeper root exploration by maize in the complex system, or that higher soil temperatures promoted faster root growth. Additionally, some studies have shown ethylene build-up in soils can encourage thickening of roots and reduced branching (Moss et al. 1988). The better aerated

soils for maize in the complex system may have contributed to both the deeper exploration, and a lower resource demand for creating the root system. A previous study that measured root length, rather than root mass, found that maize grown in the more complex system had higher root lengths in the 10-20 cm depth range compared to maize in the simple system (Lazicki et al. 2016), again suggesting that the root system of maize in the complex rotation achieved a more efficient root system with less resource investment.

A greenhouse study took soil from the two rotation treatments' plots used in the present study and homogenized it to remove structural differences (Bay et al., 2021). The researchers found differences in maize roots even after soil homogenization. In particular, maize grown in soil from the complex rotation had deeper and thinner roots compared to maize grown in soil from the simple system, while simultaneously having less root biomass. This corroborates our findings from the present field-based study, and indicates the effect is at least in part biological or chemical. Growing maize in sterilized soil would aid in parsing these effects, and certainly merits further investigation. Process-based models could also aid in supporting or refuting the hypothesis of 'deeper and cheaper' roots in more complex rotations positioning maize crops to be better buffered against unfavorable growing environments.

5 Conclusion

This study provides novel evidence that growing maize in complex rotations can result in changes to maize root structure, such as the rooting depth, as well as the investments made in root systems. The changes in root structure may affect below-ground resource acquisition by the crop, which may in turn result in higher maize grain yields under certain conditions. Differences

in penetration resistance and early season moisture may contribute to the altered root structures and plant resource investments, but there are likely additional biological and/or chemical drivers. More research is needed to understand the contributions of physical, biological, and chemical characteristics of soil in simple and more complex rotations to these changes.

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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

Figure S2 - Maximum rooting depth by year fitted values

Figure S3 - Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain weight

Figure S4 – Root to shoot ratios for 2019 and 2020

Figure S5 - Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference

498 **Figure S6** - Soil moisture at 30 and 45 cm depths in the maize phase of the complex (dark blue)
499 and simple (pink) rotations; points represent individual sensor values, lines the estimated values,
500 and ribbons the 95% confidence interval around the estimates

501

502 The following tables are included as supplementary material:

503 **Table S1** - Summary of ‘background’ root samples taken shortly before maize planting

504 **Table S2** - Summary of fixed effect tests on root mass

505 **Table S3** - Summary of root mass added contrasts for the complex versus simple rotation at each
506 depth

507 **Table S4** – Summary of above-ground growth-analysis and rooting depth non-linear parameter
508 fits

509

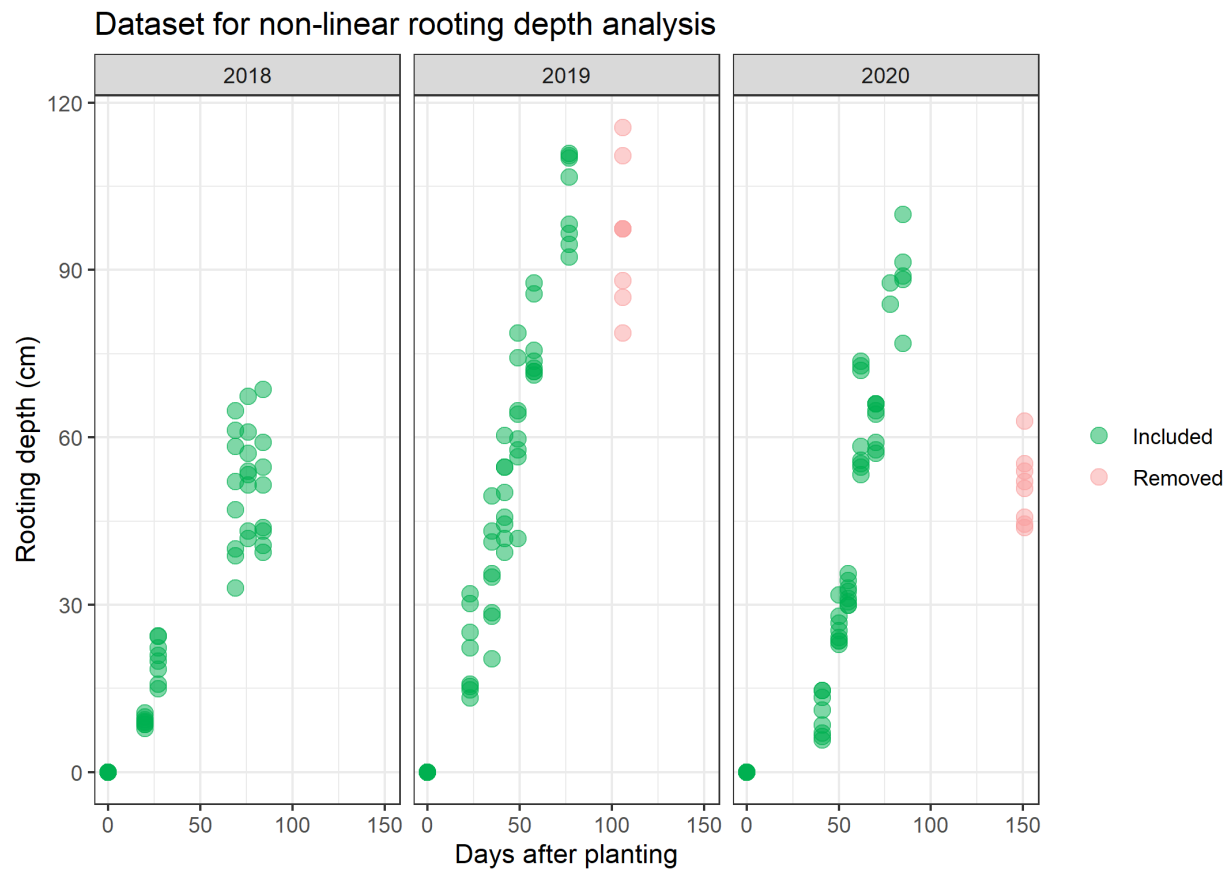
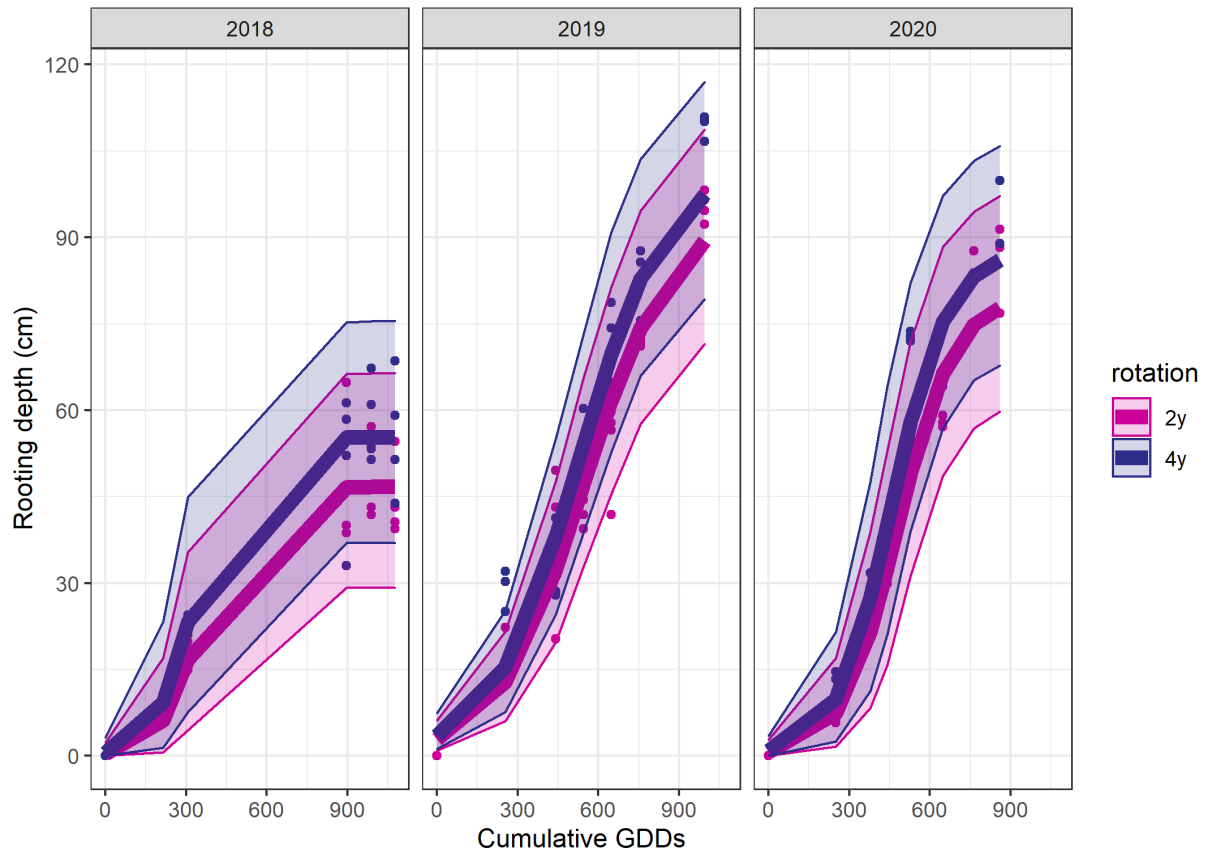


Figure S1. Rooting depth over time dataset.



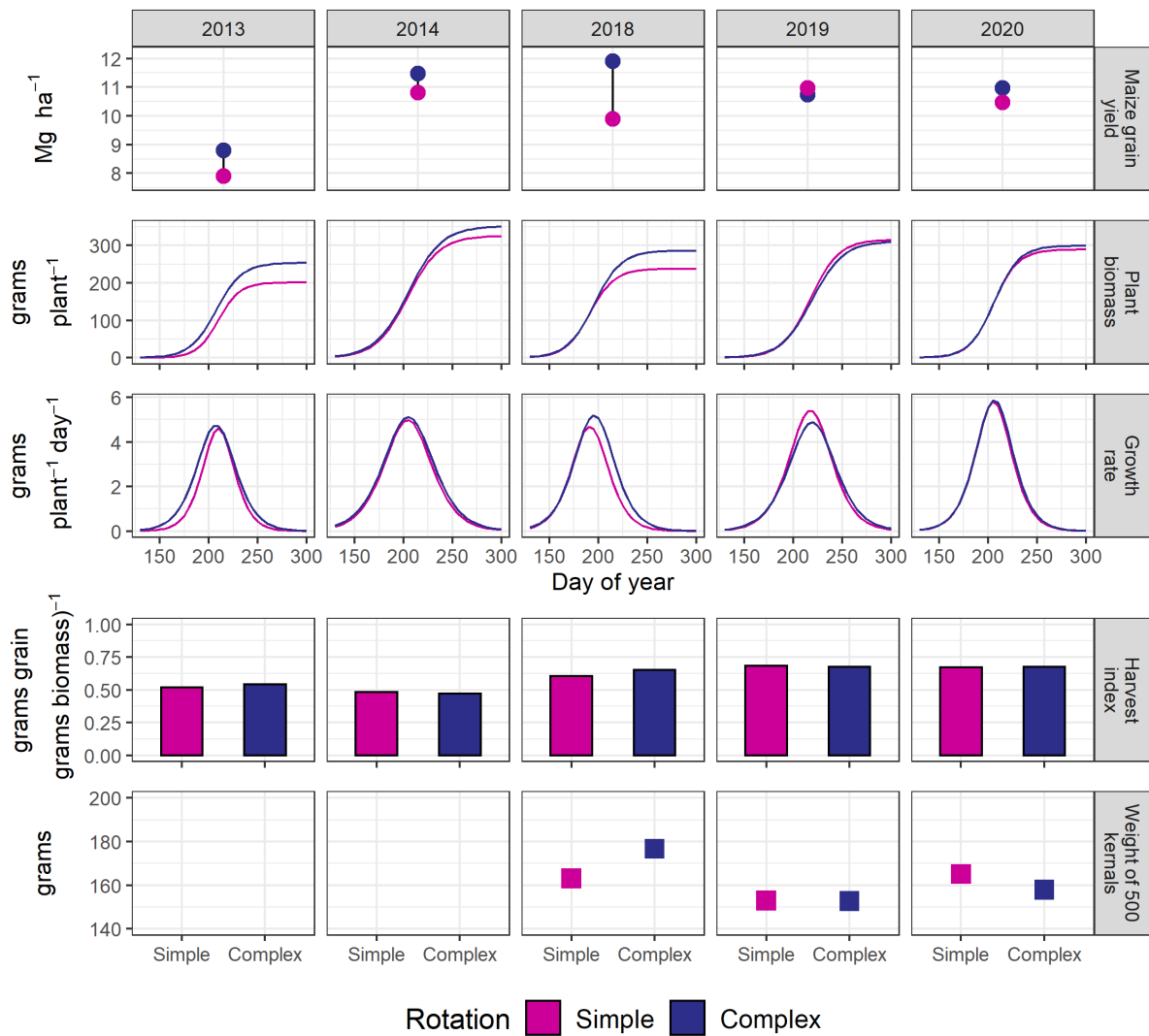
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514 *Figure S2. Model estimates for rooting depth by year with shading representing 95% confidence*

515 *intervals without predictive smoothing*

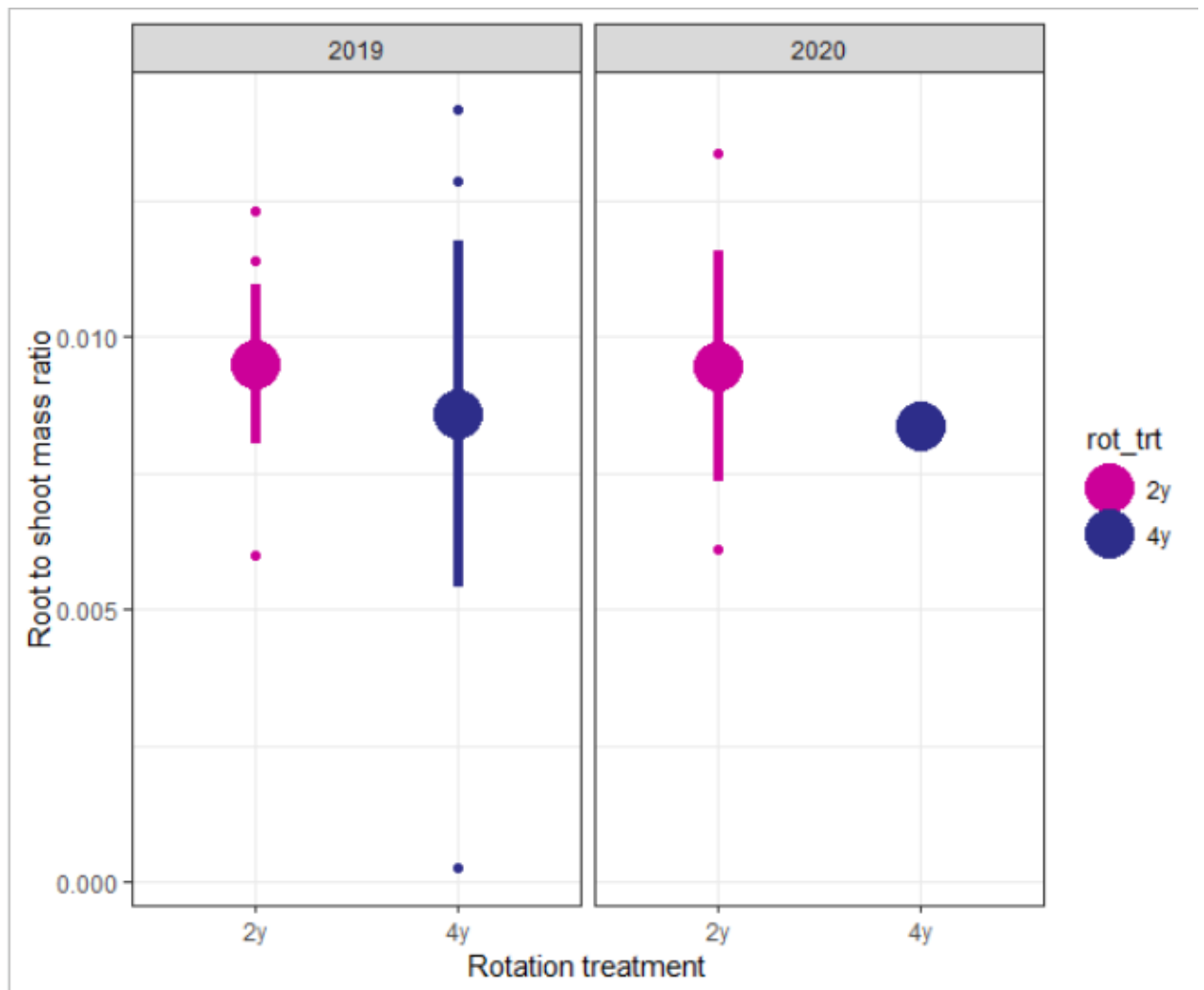
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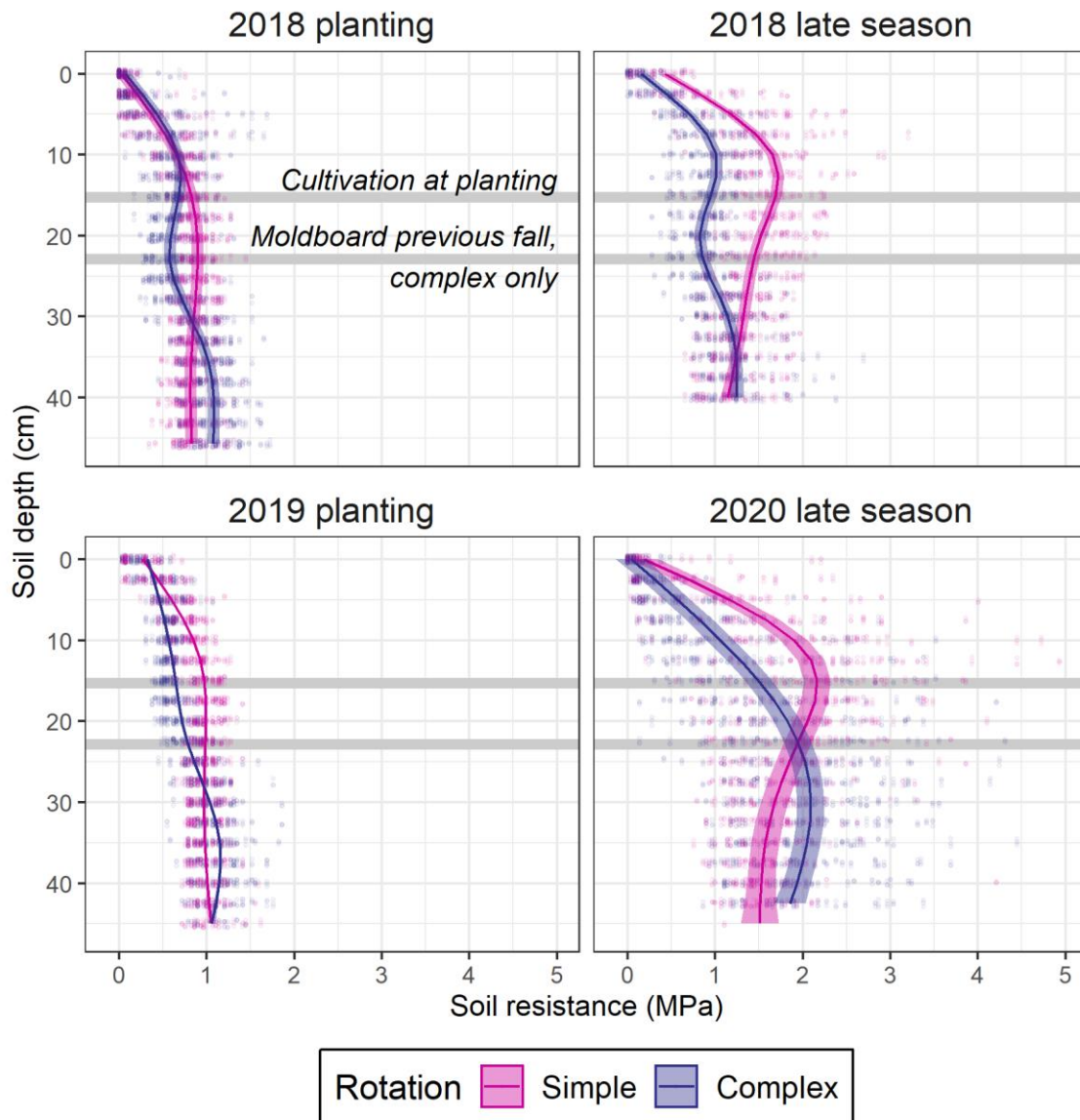
519 *Figure S3. Yields, biomass over time, growth rate over time, harvest index, 500-kernal grain*520 *weight*



521

522 *Figure S4. Root to shoot ratios for 2019 and 2020, with means and standard errors presented as*
 523 *large points and vertical lines*

524



525

526 *Figure S5. Soil penetration resistance by depth at various sampling points, approximate depths*527 *of tillage operations are provided for reference*

528

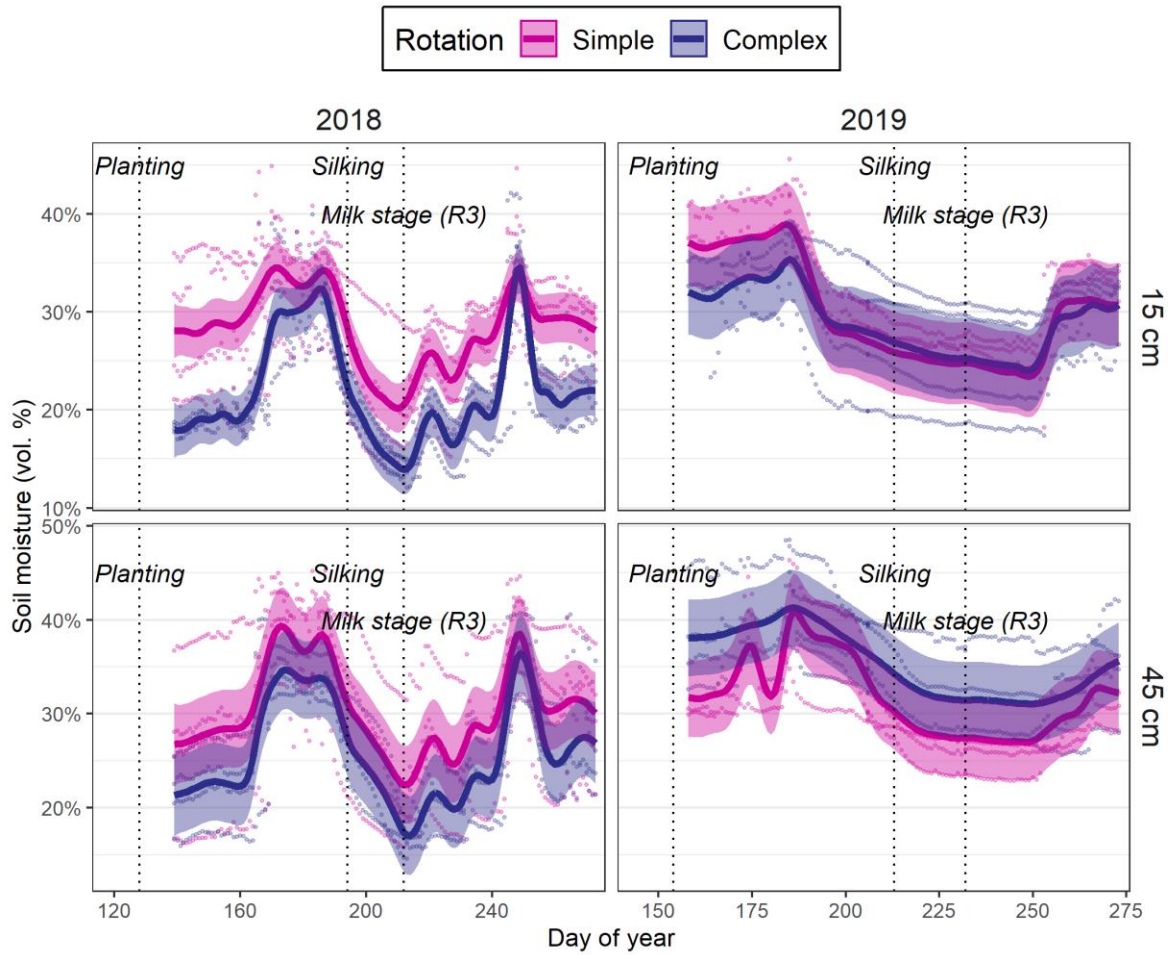


Figure S6. Soil moisture at 30 and 45 cm depths in the maize phase of the complex (dark blue) and simple (pink) rotations; points represent individual sensor values, lines the estimated values, and ribbons the 95% confidence interval around the estimates

539 **Table S1.** Summary of 'background' root samples taken shortly before maize planting

Year	Replicate	Simple Rotation	Complex Rotation
<i>Root Material (kg ha⁻¹)</i>			
2019	1	542	740
	2	158	1028
	3	119	248
	4	68	441
2020	1	2753	281
	2	136	483
	3	182	982
	4	159	2048

540

541 **Table S2.** Summary of fixed effect tests on root mass

Source	DF*	Denominator DF	F Ratio	p-value
Year	1	1.88	6.257	0.14
Rotation	1	6.21	0.127	0.73
Year x Rotation	1	6.21	0.024	0.88
Depth	3	30.65	0.6517	0.59
Year x Depth	3	30.65	1.1693	0.34
Rotation x Depth	3	30.65	4.2414	0.013
Year x Rotation x Depth	3	30.65	1.4503	0.25

*DF = Degrees of freedom

542

543 **Table S3.** Summary of root mass added contrasts for the complex versus simple rotation at each
544 depth

Depth	Numerator DF*	Denominator DF	F Ratio	p-value
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

*DF = Degrees of freedom

545

546 **Table S4.** Summary of 3-parameter logistic curve fits for above-ground growth-analysis and rooting
547 depth analysis

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

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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.
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