ARE ROOTS THE DRIVERS OF THE ROTATION EFFECT IN MAIZE?

Virginia Nichols1, William Osterholz2, Sotirios V. Archontoulis3, Matt Liebman3

1Swette Center for Sustainable Agriculture, Arizona State University, Tempe, Arizona, USA

2Soil Drainage Research Unit, USDA ARS, Columbus, Ohio, USA

3Department of Agronomy, Iowa State University, Ames, Iowa, USA

## Abstract

It is well-established that maize (*Zea mays* L.) grown in complex cropping systems can require less 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. To examine the possible role of maize roots in the rotation effect 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. Additionally, we measured soil penetration resistance and soil moisture, and performed a growth analysis on aboveground maize biomass. From 2013-2020, maize grain yields averaging 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 approximately 100 days after planting, maize grown in the complex system added significantly less root mass (436 kg ha-1) than the maize grown in the simple system in the surface soil layer (0-15 cm), but the two systems’ root investments did not differ below that depth. Soil penetration resistances from 0-30 cm were 30% lower in the complex versus simple system. Early in the season, the complex system also consistently had drier soils compared to the simple system. We posit 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. It is possible that this ‘deeper and cheaper’ root layout provided resilience against un-favorable growing conditions, regardless of their timing, leading to increased resource acquisition and significantly higher grain yields in some years. Process-based models could aid in supporting or refuting this hypothesis, and greenhouse experiments could indicate whether the root differences observed in the field reflect chemical, physical, and/or biological characteristics of the soil. To our knowledge, this is the first report of cropping system complexity impacting maize rooting depth. Similar measurements taken in other Midwestern locations would aid in understanding the role of maize rooting depth in the rotation effect in other contexts.

## Introduction

In the Midwestern United States (US), a substantial portion of agricultural land is dedicated to maize-(*Zea mays* L.) based systems (Boryan et al., 2011; USDA National Agricultural Statistics Service Cropland Data Layer, 2021). Over the past 60 years the diversity of these maize systems has been reduced from multi-species rotations that included small grains and forage legumes to maize monocultures or simple alterations of maize and soybean (*Glycine max* [L.] Merr) (Aguilar et al., 2015; Hijmans et al., 2016; Crossley et al., 2021). Several undesirable effects 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 would enable 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) 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. As maize cropping systems move from monocultures to simple rotations, and simple to extended rotations, maize yields 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 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 strong evidence it is due, at least in part, to yield suppressive mechanisms in the monoculture system, potentially due to soil biological conditions constraining root development (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 distributions of resources, microbial communities, and nutrient cycling activity in 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), but it is unclear how these altered distributions translate to higher maize yields in the complex rotations. A recent study showed maize root-associated bacterial and fungal communities differed in simple versus complex maize systems, with a greenhouse study suggesting these differences are associated with different root architectures (Bay et al. 2021). There is evidence that suggests there are differences in maize root distributions in the field (Lazicki et al., 2016), but the data is limited to shallow depths and does not control for previous crop root carryover. It is feasible that, similarly to the continuous maize penalty, differences in maize root structures are contributing to increased resource capture in complex rotations, thus driving higher maize yields compared to the simpler system.

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

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 above ground maize growth analysis throughout five seasons, soil penetration resistance measurements (2018-2020), and soil moisture measurements (2018-2019).

## Methods and Materials

### Sampling

Treatments consisted of two maize-based rotations: 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 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.

Table Summary of rotation managements

|  |  |  |  |
| --- | --- | --- | --- |
| **Rotation** | **Crop sequence** | **Nitrogen sources** | **Tillage regime** |
| Simple 2-year | Soybean-  **Maize** | Mean total of 180 kg ha-1 mineral 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 cultivation preceding maize planting |
| Complex 4-year | Soybean-Oats/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 field cultivation preceding soybean planting, no tillage events preceding oat/alfalfa planting, fall moldboard plowing of the alfalfa crop followed by spring discing and field cultivation preceding maize planting |

Each phase of the rotation treatments was present every year in four replicate blocks within a 9-hectare experiment established in 2001 at the Iowa State University Marsden Farm in Boone County, Iowa. Weather data was collected from a weather station approximately one kilometer from the field site (Iowa Environmental Mesonet, 2021). Maize grain yield was measured in 2013-2020, and additional measurements (Table 2) were taken in select years during that period.

### 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 α=0.05 unless noted otherwise. In all cases several 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.

#### Grain yields, harvest indices, grain weights

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.

Table ‑ 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. |
| Full soil nutrient test | 2018 | Soil samples from 0-90 cm were analyzed in 30 cm increments for macro- and micro-nutrients. Six soil samples from each replicate were taken immediately following planting and combined for analysis. |
| Maize above-ground biomass | 2013, 2014, 2018, 2019, 2020 | Maize above-ground biomass was measured periodically throughout the season. Eight plants were cut at ground level, separated into leaf, stem, and reproductive components, dried at 60⁰C 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 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) |

Differences in harvest indices and 500-grain weights 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.

#### Rooting depth

We model rooting depth as a function of the cumulative maize growing-degree-days (GDDs) accrued since planting (base temperature 10⁰C, maximum temperature 30⁰C) 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 S6-1). 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

where *rootdepth(GDD)* is the maximum rooting depth at a given cumulative GDD after planting and *Asym*, *xmid*, and *scal* are estimated parameters. *Asym* represents the maximum rooting depth achieved, *xmid* represents the cumulative GDD value at which half of the maximum rooting depth is 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 *Asym*, *scal*, and *xmid*; a random effect of a year-factor on *Asym*, *xmid*, and *scal*; a random effect of block on *Asym*; and a power variance structure.

#### Root mass

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). 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 1‑2) 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. 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.

#### Growth analysis

Above-ground biomass as a function of day-of-year was modelled separately for each rotation in each year using a three-parameter logistic curve (Eqn. 1). A model using GDDs produced similar results, but we chose to report the model using calendar days for ease in interpretation. We chose a three-parameter logistic curve over other nonlinear models (Archontoulis and Miguez, 2013)??? based on AIC criteria. 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 R.

#### 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.

#### 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*.

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## Results

### Grain yields and weather

Maize yields ranged from 7.0 to 12.7 Mg ha-1over 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 Mg ha-1, while maize grown in the complex rotation yielded 8% higher with a mean grain yield of 11.0 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 19⁰C. 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.

Chart, line chart

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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 with lines connecting points for ease in viewing and significantly different yields (p<0.01) indicated with stars 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 complex system’s maize mean yield advantage over the simple system and point color representing the measurement set for that year.

### Rooting depth and root mass

The rooting depth of maize grown in the complex rotation trended consistently deeper at most sampling times in all three growing seasons (**Figure 2**). Rotation affected maximum rooting depth (*Asym*; 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 (*xmid*; p=0.19; **Table S1**).

Chart, line chart

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Figure 2. Maximum maize rooting depth across three growing seasons (2018, 2019, 2020) in simple (2-year, pink) and complex (4-year, dark blue) rotations; lines are provided to aid in visual interpretation only, measurement errors are omitted for simplicity but are available in supplemental material; first points at 0 depth indicate maize planting date.

The effect of rotation treatment on root mass differed significantly by sampling depth (p=0.01). Year and its interactions did not have significant effects (**Table S2**), so results are presented as marginal estimates over years (**Figure 3**).

Chart, box and whisker chart

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Figure 3. Root mass added (root mass at maturity less root mass present at planting) at each soil depth

for maize grown in the simple (2-year rotation, pink) and complex (4-year rotation, dark blue) systems, bars represent marginal means and vertical lines the standard error of the mean.

At the 0-15 cm depth increment, the root mass added in the two systems differed significantly (p = 0.02), with the simple system increasing by 314 kg ha-1 (SE:113) from the baseline measurements, while the complex rotation’s root mass decreased by 122 kg ha-1 (SE:113) from the baseline measurement. At all other depths, the difference between the complex and simple system’s additions was not statistically significant (**Table S3**).

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### Growth analysis

The maximum aboveground maize biomass (asymp?) 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; Table S1**). The date at which the maize achieved half of its maximum biomass (*xmid*) 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*) 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.

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Year†** | **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** |
|  | *Ratio* |  |  |  |
| 2018 | 1.20\*\*\* | Late season\*\*\* | 1.08\*\*\* | 1.13\*\* |
| 2013 | 1.10\*\* | Early season\*\* | 1.04\*\* | *-* |
| 2014 | 1.06\* | ns | ns | *-* |
| 2020 | ns | ns | ns | ns |
| 2019 | ns | ns | ns | ns |
| †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 | | | | |

### 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 S4**). 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 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.

### Soil moisture

In both years of measurement, the complex system’s soil moisture at 15 cm depth was significantly lower for the first month following planting (**Figure S5**). 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 mirrored at the 45 cm depth.

## Discussion

In this study, we tested the hypothesis that maize grown in a more complex rotation system invests less resources into root growth but achieves a root system that increases the likelihood of higher grain yields. While our data does not establish direct cause-effect relationships, it is consistent with this hypothesis. In the two years of root mass measurement, there was a greater increase in root mass at 0-15 cm depth in the simple compared to the complex rotation, and no differences were detected between systems at greater depths. This indicates the investment in total maize root mass to 60 cm was greater in the simple rotation than in the more complex rotation. There was no significant effect of year on the differences in root mass, suggesting the root differences may be consistent across years. Additionally, maize in the complex rotation achieved a deeper maximum rooting depth in all three years of measurement, which covered widely ranging environmental conditions (**Figure 1**).

While the lower investment in shallow roots and increase in maximum rooting depth were consistent characteristics of maize grown in the more complex rotation, these differences did not always correspond with higher grain yields – i.e., not all growing season dynamics offer a yield benefit to ‘steeper and cheaper’ roots. However, the fact that the maize growth advantage was not consistently timed in the years when there was a large rotation effect on maize yield (**Table 3**) suggests this root layout may provide the plant with resilience against unfavorable conditions, regardless of their timing. Again, while our dataset is not conclusive, it is consistent with our hypotheses and provides novel new information that enriches our knowledge base on the rotation effect. Thus, 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 un-favorable growing conditions and produce higher grain yields compared to the maize grown in the simpler rotation. 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).

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 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 (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). Neither system had resistances high enough to meaningfully impede root penetration, but the differences (0.1-0.4 MPa) are 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). Additionally, 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. This is consistent with the lower bulk densities of the complex-rotation soils reported in previous studies (Baldwin-Kordick et al., 2022). 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 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 the physical effects (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 indicates the effect is at least in part biological or chemical, and supports our findings in the field. 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.

## Conclusion

This study provides novel evidence that growing maize in complex rotations can result in changes to maize root structure. These changes may affect below-ground resource acquisition by the crop, which may in turn result in higher maize grain yields under certain conditions. More research is needed to understand the contributions of the soil’s physical, biological, and chemical characteristics in simple and more complex rotations to these changes.

## 

Acknowledgments

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## Appendix. Supplementary Material

The following figures are included as supplementary material:

Figure S1 – Maximum rooting depth over time 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 - Soil penetration resistance by depth at various sampling points, approximate depths of tillage operations are provided for reference

Figure S5 - 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

The following tables are included as supplementary material:

Table S1 - Summary of growth-analysis curve fits

Table S2 - Summary of fixed effect tests on root mass

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

Chart, scatter chart

Description automatically generated

Figure S1. rooting depth over time dataset.

Chart, histogram

Description automatically generated

Figure S2. Model estimates for maximum rooting depth by year

Diagram

Description automatically generated

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

Chart

Description automatically generated with low confidence

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

Chart, histogram

Description automatically generated

Figure S5. 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

Table S1. Summary of growth-analysis curve fits. Write the model here

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Year** | **Rotation** | **Asym** | **xmid** | **scal** |
|  |  | *(cm)* | *(GDDs)* |  |
| 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) |

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* |  |  |  |  |

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