Introduction

Soils store a huge amount of carbon (C) and that’s important. The interactions of soil characteristics such as clay and mineral content with climate conditions such as temperature and precipitation and vegetative properties such as primary production play a strong role in how and how much plant matter transforms from living tissue into a component of the soil matrix. The nature of these interactions is less well known as we move deeper into the soil. Indeed, when it comes to the formation and stabilization of soil organic matter, we are theory-rich and data-poor, especially below the top 20-30 cm. Much remains unknown about the development of the vertical distribution of soil organic matter.

Subsoil organic matter consists of root biomass, dissolved organic matter, and transported particulate organic matter. However, the relative importance and factors controlling the contribution of these C sources is not well known. **Ecosystems consistently show disproportionately less soil C at the surface and more soil C at depth when compared to root biomass. Half of SOC is below 20 cm, but only 1/3 of the total roots.**

There is evidence that the contribution of microbial-derived organic matter increases with depth, but the mechanisms behind this contribution are unclear and quantitative data is lacking.

Mollisols are soils that formed under grasslands in semi-arid to semi-humid mid latitudes and are characterized by a deep (60-80 cm), organic matter-rich surface horizon. They are typically base rich and often calcareous. Mollisols support the world’s most agriculturally productive regions, meaning that most mollisols have experienced a shift away from the perennial grasslands under which they formed to annual plant agroecosystems which regularly experience soil disturbance and differ significantly in quality and quantity of root biomass inputs. Replacing natural ecosystems with agricultural systems initially led to major decreases in surface organic matter through losses by erosion and increased microbial metabolism. Less well known is the effect of ecosystem changes on subsoil organic matter.

We examined the belowground reconstruction of native vegetation on a Mollisol after >100 years of annual cropping to gain a unique perspective on characteristics of root inputs that would not necessarily be noticed in established prairie systems, but that contribute to dynamics of the belowground ecosystem. We compared the root inputs of a reconstructed prairie system to the root inputs of a maize cropping system and asked the following questions: 1) How long after reconstruction does a prairie root system begin to contribute to organic matter? 2) How does the quantity, distribution, and quality of root biomass differ in native perennial and non-native annual ecosystems? 3) What do these differences in inputs tell us about the historical belowground ecosystem under which these soils developed in comparison to the systems under which these soils continue to change?

2 Materials and Methods

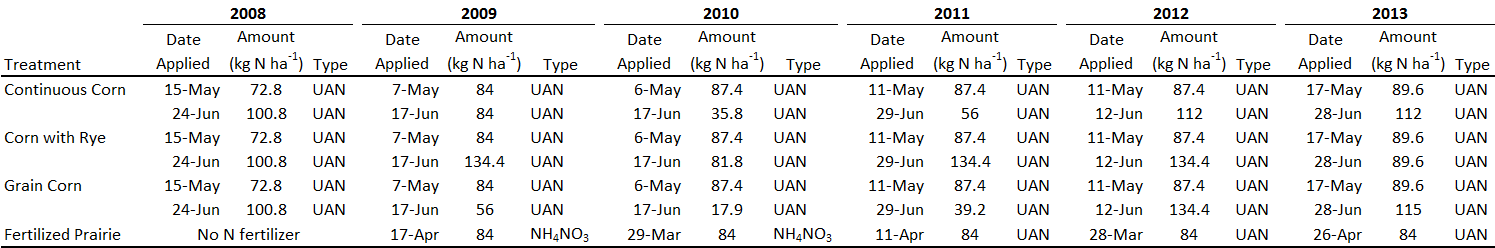
*2.1 Site Conditions and Experimental Design*

We conducted the experiment in Boone County, IA, USA on the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55′N, 93°45′W). Soils at the site were primarily Webster silty clay loam (ﬁne-loamy, mixed, superactive, mesic Typic Endoaquoll) and Nicollet loam (ﬁne-loamy, mixed, superactive, mesic Aquic Hapludoll). The 60-year mean growing season precipitation 11 km from the site was 720 mm. Prior to initiation of the ﬁeld experiment in 2008, the site was used for maize and soybean production and was planted with soybean in 2007. Soil sampling to 15 cm in November 2007 indicated mean soil pH was 6.7, mean organic matter concentration (via dry combustion analysis with a conversion factor of 1.724 from total carbon to organic matter [Schumacher 2002]) was 51 g kg-1, mean extractable phosphorus concentration (via Bray-1 procedure) was 11 mg kg-1, and mean extractable potassium (via Mehlich-3 procedure) was 141 mg kg-1.

Experimental plots were 27 m x 61 m and were arranged as a spatially balanced complete block design (van Es *et al*., 2007). Five cropping systems were studied: a corn-soybean rotation with annual grain removal (hereafter corn-soybean rotation), continuous corn with annual grain and stover removal (hereafter continuous corn), continuous corn with grain and stover removal and rye used as a winter cover crop (hereafter continuous corn with rye), reconstructed multispecies prairie with annual aboveground biomass removal (hereafter unfertilized prairie), and N-fertilized reconstructed multispecies prairie with annual aboveground biomass removal (hereafter fertilized prairie). All of the treatments were managed without tillage. Conventional farm machinery was used for planting, fertilization, crop protection, and harvest operations. Herbicides were not used in the prairie systems except for a small number of spot treatments for Canada thistle (*Circium canadense*) control, and the timing and frequency of herbicide use in the annual cropping systems varied among treatments. Nutrient management also varied among all treatments (Table 1).

Both prairie treatments were sown on 19 May 2008 with the same custom seed mix obtained from Prairie Moon Nursery (Winona, MN, USA) that contained 31 species, including C3 and C4 grasses and leguminous and non-leguminous forbs (Table S1). All species were perennial and sourced from within 240 km of the experiment site. The composition of the seed mix by weight was 12% C3 grasses, 56% C4 grasses, 8% legumes, and 24% non-leguminous forbs. A detailed description of the prairie plant community compositions can be found in Jarchow and Liebman (2013). The fertilized prairie treatment received no fertilizer in 2008 (the establishment year), and was fertilized at a rate of 84 kg N ha-1 year-1 in all subsequent years. This fertilizer rate was chosen because it was similar to the maximum rate of pre-planting N fertilization recommended for maize (Blackmer *et al*., 1997) and the expected N removal in the harvested biomass of perennial grasses grown in the area (Heggenstaller *et al*., 2009). Fertilization timing can be found in Table 1.

The maize used was a 104-day relative maturity hybrid with transgenes for glyphosate resistance, corn borer (*Ostrinia nubilalis*) resistance, and corn rootworm**(***Diabrotica* spp.) protection (Agrigold 6325 VT3). Maize was planted following standard practices (Abendroth *et al*., 2011) in rows spaced 76 cm apart at 79,500 seeds ha-1 on 6 May 2010 and 82,500 seeds ha-1 on 11 May 2011. Fertilizer rates and types can be found in Table 1. Rates of N added after planting were based on results of late-spring tests of soil nitrate-N concentration (Blackmer *et al*., 1997). All N was applied as urea-ammonium nitrate (32% N). An unfertilized maize treatment was not included in the experiment because the effects of N fertilizer on maize have been extensively studied and modeled, with N fertilization leading to greater biomass and higher grain yields (Cerrato and Blackmer 1990; Sawyer *et al*., 2006).

Table 1. N fertilizer amount, type, and date applied for all COBS treatments.

*2.2 Data Collection*

*2.2a Soil Collection*

Soil cores were taken to 1 m depth in all plots each year using a hydraulic soil probe (Giddings Machine Co., Windsor, CO, USA) after all crops were harvested. Sampling occurred by replicate block from 31 October-25 November 2008, 9-11 November 2009, 25-28 October 2010, and 28-31 October 2011, 16-17 October 2012 and 7-11 October 2013.

In 2008, two cores were taken per plot. A 0-30 cm fraction was taken with a 10.2 cm internal diameter soil probe; the 30-100 cm fractions of the cores was taken within the same hole as the 0-30 cm fraction, but with a smaller soil probe. In Blocks 1 and 4, the internal diameter of the core was 6.0 cm. In Blocks 2 and 3, the internal diameter of the core was 5.2 cm.

In 2009 and 2010, four cores were taken per plot. The 0-30 cm fraction of the cores was taken with a 10.2 cm internal diameter soil probe; the 30-100 cm fraction of the cores were taken directly below the 0-30 cm fraction with a 5.1 cm internal diameter probe. In 2011-2013, four cores were taken per plot, and the entire core was taken with a 5.1 cm internal diameter probe.

Soil cores were ultimately divided into three or five depth increments. In 2008, depth increments were 0-30 cm, 30-60 cm, and 60-100 cm. In 2009-2013 depth increments were 0-5 cm, 5-15 cm, 15-30, cm, 30-60 cm, and 60-100 cm. Following division and extraction from the field, soil cores were stored at 5°C until processing was initiated.

*2.2b Root Pool Collection*

Root extraction from the soil began by washing the soil samples in wire mesh tubes (0.28 mm mesh) for 3 h in an elutriator (Wiles et al., 1996). Roots were removed from the remaining soil by suspending the air-dried sample in water and collecting the roots, which floated, with sieves followed by manually removing any remaining non-root material that was present in the samples. Any plant crowns that were present in the samples were removed and were not considered to be root biomass. Roots were then dried at 70°C for at least 4 h before being weighed. All above- and belowground biomass samples were ground to 2 mm with a centrifugal mill and concentrations of C and N were determined by combustion analysis at the Soil and Plant Analysis Laboratory at Iowa State University (Ames, IA, USA).

Each year before roots were washed, 60-100 g of root-free soil was removed from each depth increment, air-dried, and archived in airtight containers at room temperature. In 2008 and 2013, this soil was ground on a roller-mill and organic C content was determined by catalytic oxidation and CO2 measurement with NDIR in an Elementar TOC Cube at Brookside Laboratories, Inc. (New Bremen, Ohio).

*2.2c In-season Root Growth*

In 2010 and 2011, root biomass was measured with an in-situ growth core approach (Neill 1992) to capture only those roots growing within the measurement year. After fall harvest in 2009 and 2010, eight 10.2-cm-diam soil cores were taken to 30 cm depth in each plot and brought to the laboratory. Holes created in the field were held open during the winter by capped 10.2 cm PVC piping. In the laboratory, cores were divided into 10 cm sections and virtually all roots were removed by hand. Soil was stored in intact cores at 30°C for the first year of the experiment and 4 °C in sealed plastic bags for second year of the experiment. The differences in storage conditions did not have an apparent effect on the outcome of the experiment. At the end of winter while plants were still dormant, the root-free soil was returned to its original location in the field in 10 cm depth increments. Soil was packed to imitate the surrounding bulk density, approximately 1.4 g cm-3. Root-free zones were located randomly within prairie plots and at 20 cm from maize rows. Eight root-free areas were situated within each plot, allowing duplicate sampling at four time points throughout the growing season. Two 4-cm-diam soil cores were taken within each 10.2-cm-diam root-free area to a 30 cm depth at each root sampling date. Bulk soil was washed from the roots with water using a soil elutriator (Wiles *et al*., 1996), roots were dried at 60° C for 24 hours, non-root biomass was removed from the roots by hand, and roots were weighed.

In-situ growth cores have a few disadvantages. Belowground biomass measurements from in-situ cores capture only lateral roots, leading to overall root biomass values that are lower than measurements that may include vertical roots. Root-free soil also may create some experimental artifacts by providing a zone free of competition that may encourage more root growth. However, any method of measuring root growth in-situ has disadvantages.

*2.3 Data Analysis*

Root pool mass for the entire meter depth was calculated by summing together the root mass for each depth increment of an entire core and whole core root masses between treatments were compared within each year using contrasts within a linear mixed effect model in R. Treatment differences within depths and depth differences within treatments were also made for POXC using contrasts within a linear mixed effects model in the R package *nlme* (Pinheiro et al. 2013). Treatment differences within depths within years and differences between treatments within depths within years for root biomass were also made with contrasts with linear mixed effects models, but proc glimmix in SAS (SAS Institute, 2011) was used.

Because root mass in 2008 was measured at three increments (0-30 cm, 30-60 cm, and 60-100 cm) instead of five increments (0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm, and 60-100 cm), 2008 root mass for 2008 0-5 cm, 5-15 cm, and 15-30 cm was estimated by multiplying the average 2009-2013 proportion by the 2008 0-30 cm increment. No important comparisons were made using this estimated data, but the data was used as a starting point for graphing C:N ratios in different depth increments and fitting curves to root accumulation. C:N ratios were compared between treatments within years within depths and between years within treatments within depths using proc glimmix in SAS.

Root mass measured at the end of the each growing season was subset by depth increment and each subset was fit by both a logistic model and a linear model for each plot. Logistic models and linear models were compared against each other using Akaike’s Criterion (AIC) and the model with the lowest AIC was chosen. The AIC was not vastly different for any of the comparisons, but the logistic model had the best fit for every depth (Appendix Figure 1). Model fits and comparisons were done using the R package *nlme* (Pinheiro et al. 2013).

The first derivative of the logistic model was used to calculate the daily rate of accumulation. Parameters from the logistic model were used to predict both amount and rate of accumulation for each day for each depth in each plot of the experiment. These predictions were averaged for each treatment and plotted. The annual mean rate was calculated by averaging accumulation rates across each growing season for each depth in each plot. Comparisons of rates between treatments within depths and within years and comparisons of rates between depths within treatments within years were made with proc glmmix in SAS.

In-situ root measurements in 2010 and 2011 combined with differences in root pool masses at 30 cm over these years were used to calculate a root turnover constant (k) and root mean residence time (mrt) using the equations k = loss/pool and mrt = 1/k . Root pool loss during each year was calculated as the difference between the mass accumulated during that year and the gain measured by in-situ growth cores. The root mass measured at the end of the year was the pool value.

3 Results

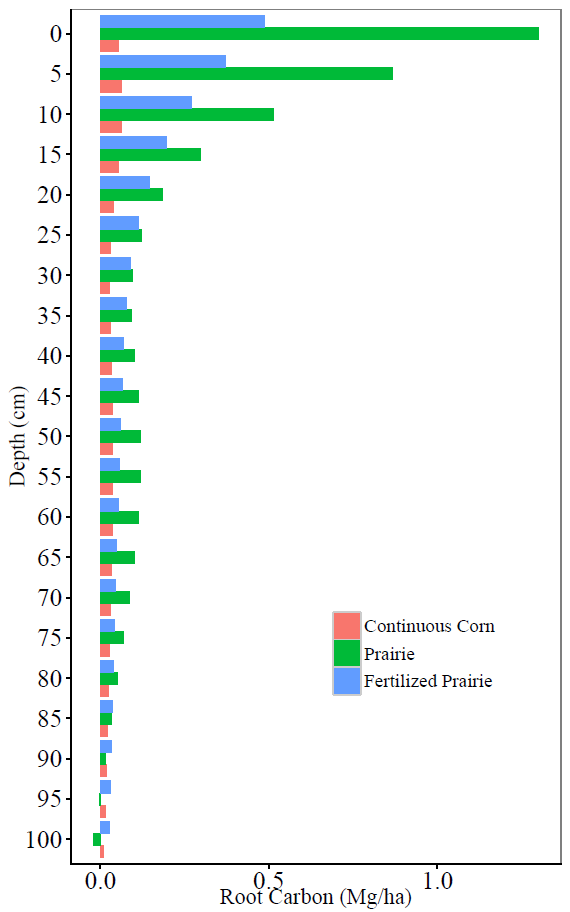


Figure 1. Absolute difference in root C pools six years after prairie establishment.

Six years after the establishment of the experiment, the unfertilized prairie root C pool was almost 6 times greater than the maize root C pool and the fertilized prairie root C pool was 3.5 times greater than the maize root C pool over a 1m depth. XX% of the unfertilized prairie root pool, xx% of the fertilized prairie root C pool and xx% of the maize root C pool was found in the top 30 cm of soil (Fig 1).

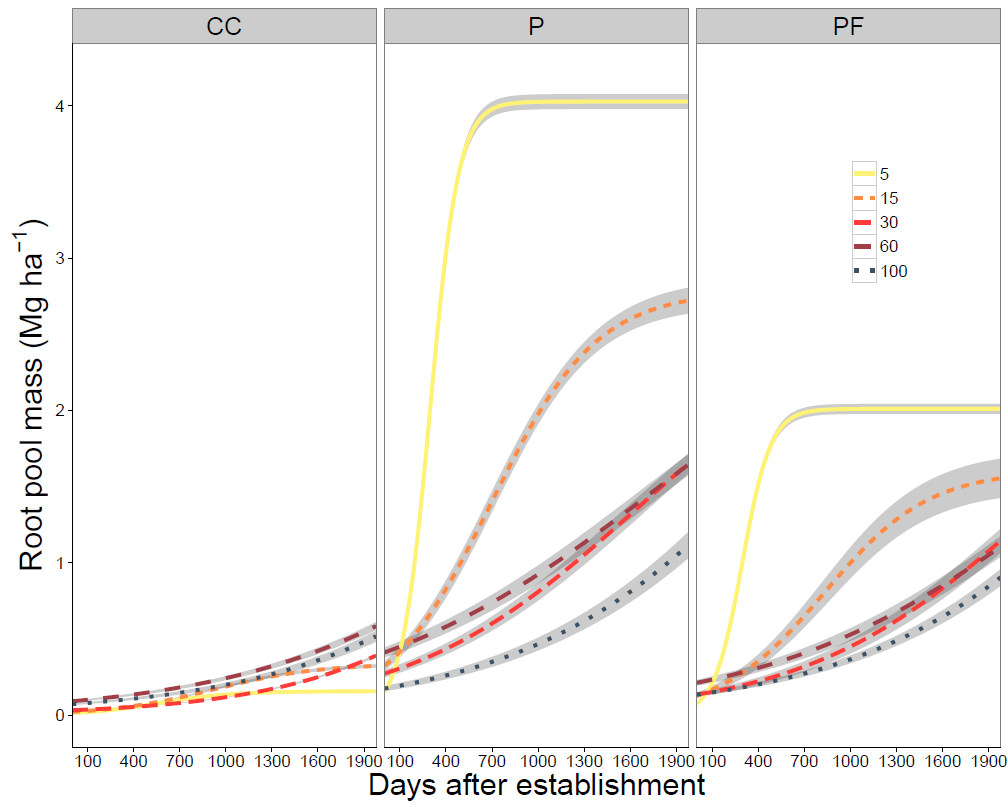


Figure 2. Accumulation of root pool mass over six years.

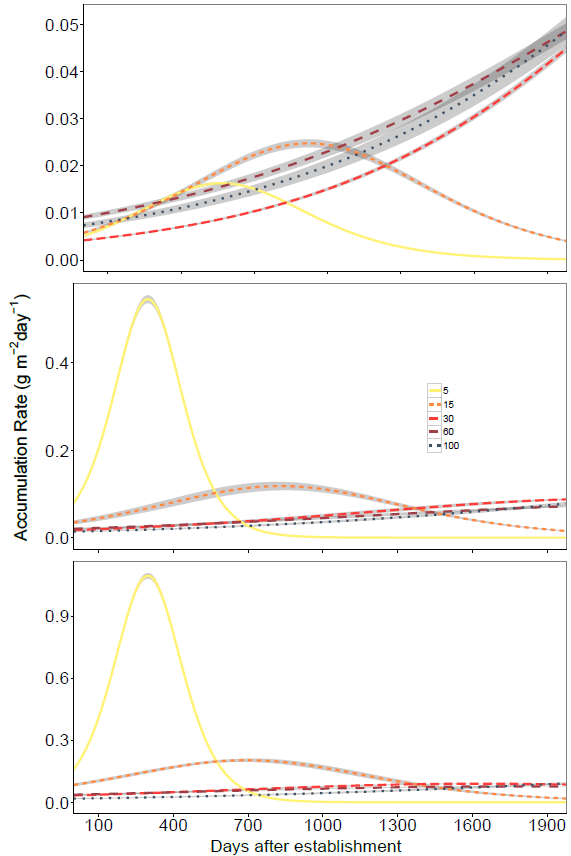


Figure 3. Rates of root pool mass accumulation over 6 years in continuous corn (top), fertilized prairie (middle) and unfertilized prairie (bottom).

Table 1. Root pool accumulation rates averaged across each growing season. Differences in lowercase letters indicate significant differences between depths within treatments within years (read up and down). Differences in uppercase letters indicate differences between treatments within depths within years (read left to right).



Prairie rooting systems were established sequentially in the soil profile from the top down. The top five cm of the root pool peaked in the first full year of growth and then reached an equilibrium during the second full year of growth with large year-to-year variability given the sensitivity of this thin surface layer to environmental conditions (Fig S1). The next soil layer, from 5-15 cm, experienced the greatest increase in root pool mass during the second full year of prairie growth, while the 15-30 cm and 30-60 cm depths didn’t reach peak rates of root pool accumulation until five and six years after establishment, with no indication of when accumulation will cease. In the unfertilized prairie, rates of root pool accumulation in the 60-100 cm of the soil in the sixth year were greater than all other depths with no sign of slowing down. Fertilized prairie also had a high rate of root pool accumulation at 60-100 cm in the sixth year with no sign of decreasing.

Maize root pool accumulation was almost always slower than prairie root pool accumulation with the exception of the top 5 cm after 2010, 60-100 cm before 2011 (not different from fertilized prairie), and a greater value in maize than unfertilized prairie at 30-60 cm in 2013. There was no difference in root pool accumulation among depths until 2011 when accumulation below 15 cm began to exceed accumulation above 15 cm.

Table 2. Root turnover at 0-30 cm (I will probably average the two years).

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trt input gain loss pool k mrt

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Prairie2010 367 104 263 748 0.35 2.8

Prairie2011 387 78 309 758 0.41 2.5

FertilizedPrairie2010 146 62 84 231 0.37 2.7

FertilizedPrairie2011 168 55 113 342 0.33 3.0

Maize2010 56 18 38 44 0.86 1.2

Maize2011 48 16 31 47 0.67 1.5

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Prairie roots had a mean residence time (mrt) of 2.75 years in the top 30 cm of the profile when averaged across treatments and years (2010 and 2011). Maize roots turned over twice as fast as prairie roots when averaged across treatments and years (Table 2).

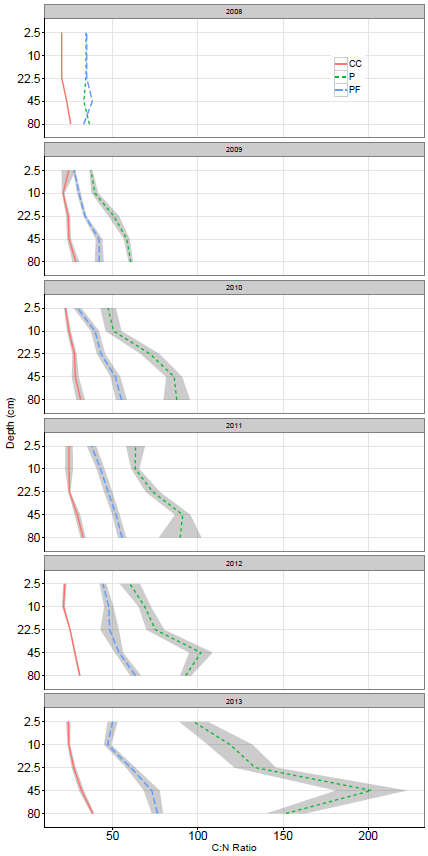


Figure 4. Root C:N ratios with depth over time.

Carbon to nitrogen ratios increased with depth in all treatments. Carbon to nitrogen ratios increased in both prairie treatments in every depth over time, although the increase in fertilized prairie was not always different between consecutive years. In all treatments, changes in C:N ratios were the result of both an increase in C content and a decrease in N content (data not shown). The maize root pool did not have in increase in C:N ratio over time.

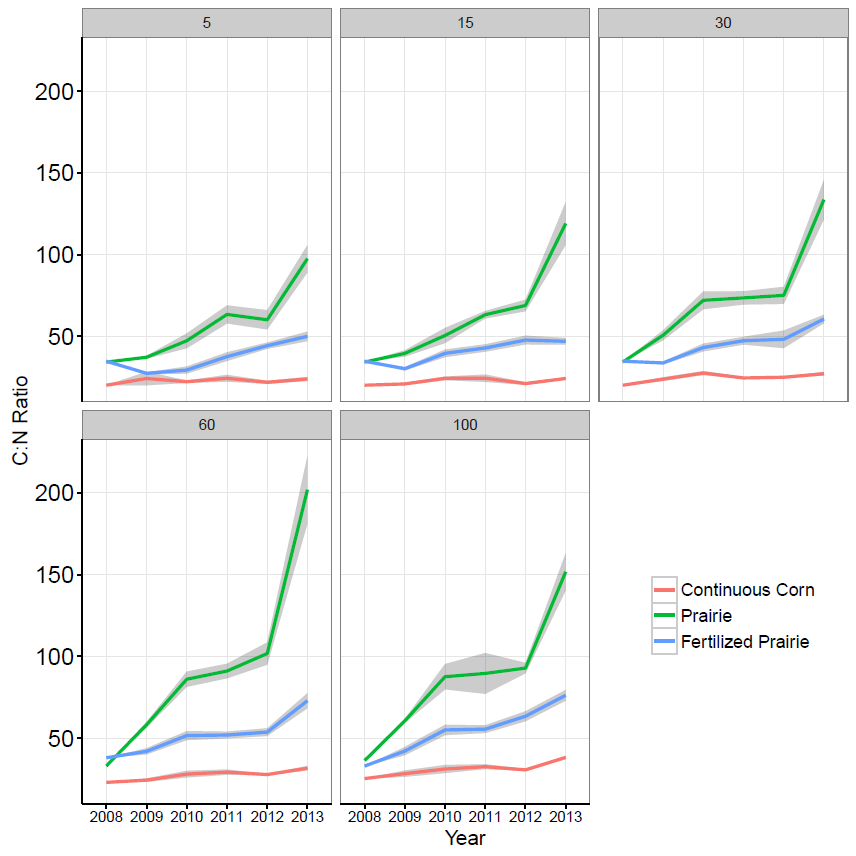


Figure 4 alternative. Root C:N ratios for each depth over time.

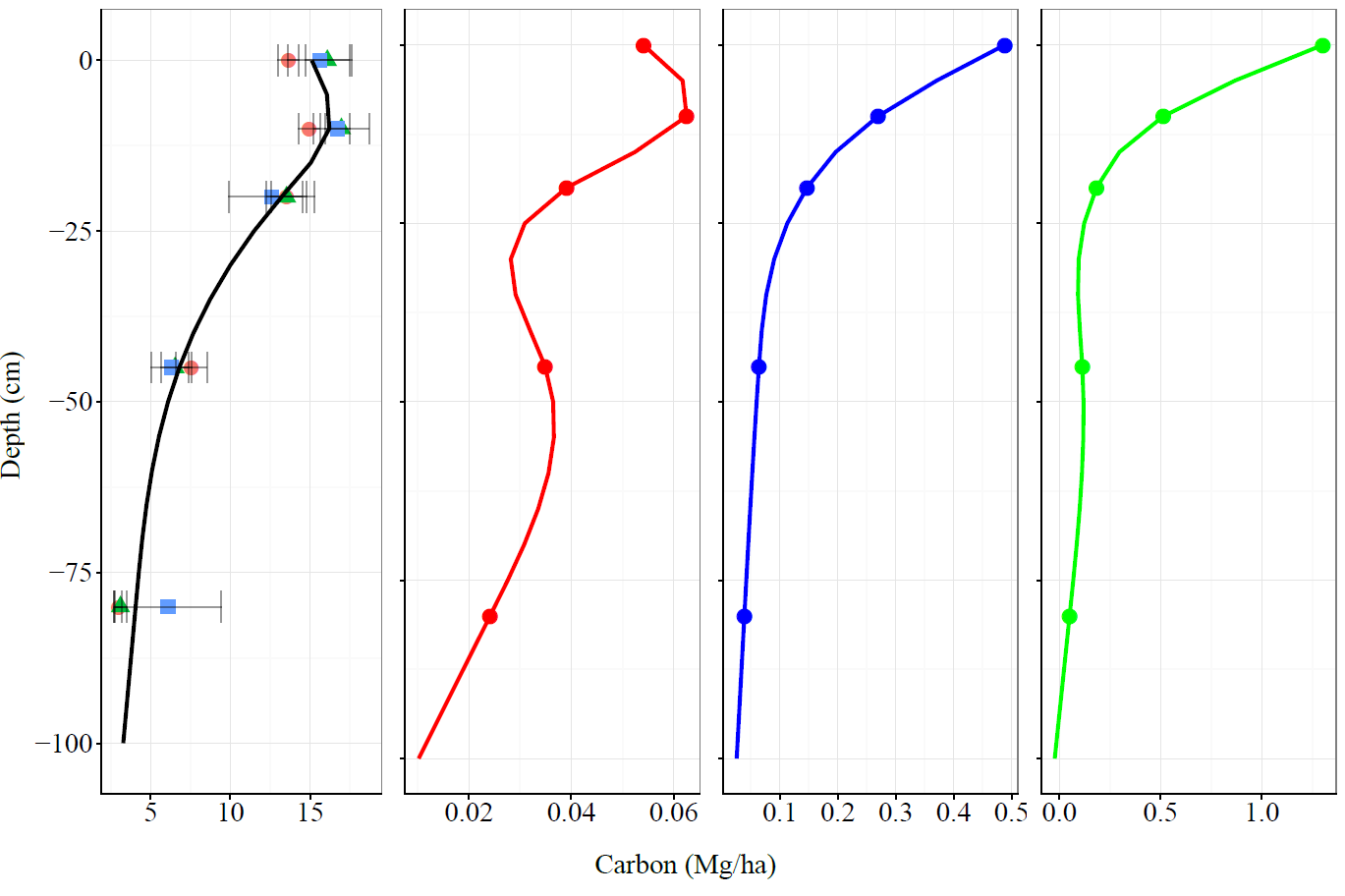


Figure 5. From left to right: total soil carbon, maize root carbon, fertilized prairie root carbon, unfertilized prairie root carbon. (Would like to have a better fit for maize)

The amount of total organic C found in the soil 6 years after establishment of the experiment was not different among treatments at any depth (Fig 4), nor was it different from initial total organic C levels (data not shown). The pattern of soil C distribution reflected the pattern of maize root distribution, not prairie root distribution (Fig 4).

4 Discussion

*4.1 Reconstruction of a native belowground ecosystem and implications for C contribution*

Because the root pool is made up of a combination of new, mature, ageing, and dead roots, its increase in mass comes from root growth, live root retention, and inhibited root decomposition. The relatively quick rate of accumulation in the top 30 cm of soil was most likely a function of new root growth, which slowed as the system became more established. Slower increases at deeper depths may be indicative that accumulation is more dependent upon the carryover of roots from previous years than at shallower depths.

Very low rates of accumulation above 30 cm indicate that roots at this depth begin consistent turnover approximately six years after establishment. An accumulation rate near zero indicates that root loss is equal to root growth and the root pool is at equilibrium. At this point, the root pool is able to begin large contributions to the soil. The mean residence time of roots above 30 cm showed that root material stayed in the root pool only 2.5-3 years, indicating a substantial potential input of organic matter to the soil

Annual root inputs were not measured below 30 cm, so turnover rates could not be calculated. However, continuous increases in the root pool at depth due to root growth and retention indicates that root tissue loss to the soil was very low during this time and the mean residence time of roots at depth may greatly exceed those closer to the surface. This means that at depth, not only are root inputs substantially lower than near the surface, but root material also becomes available to the soil much more slowly.

An increase in root C:N ratio with depth has not been previously reported in the literature and can help inform our understanding of development of the soil C profile. The turnover of higher C:N ratio root material at deeper depths would have been slower and less efficient than the turnover of root material at the surface, resulting in a lower proportion of C for stabilization in the soil relative to root tissue decomposition at shallower depths. This outcome was unexpected given that an unexplainably large amount of soil C is found at depth when compared to root distribution. However, these results are consistent with evidence that the contribution of microbial- and not root-derived C increases with depth. The finding that deep soil C accumulation is less of a function of root substrate means that it is more of a function of other factors such as rhizodeposition, microbial biomass, and environmental conditions.

Nitrogen fertilization of prairies led to a smaller root pool at every depth, with lower rates of accumulation, and lower C:N ratios. However, fertilization did not affect the time until root systems were fully established or the turnover rate of roots in the top 30 cm. Differences between fertilized and unfertilized prairie show that the pattern of distribution is a function of nutrient availability and not a response to soil space conditions because fertilized prairie used half of the space as unfertilized prairie and still showed decreased accumulation above 30 cm over time.

*4.2 Quantity, distribution, and quality of root biomass differs in native perennial and non-native annual ecosystems*

Maize root C pools are much smaller than prairie root C pools, but faster turnover times and lower C:N ratios resulted in a greater proportion of the maize root C pool available for stabilization in the soil compared to the prairie root C pool. In the top 0-30 cm, the difference in mass between even the fertilized prairie and maize is too great to be overcome by faster turnover and greater carbon use efficiency, but the difference in mass decreases with depth while the difference in C:N ratio increases and turnover times maintain the same relative relationship. This means it is possible that maize roots contribute more C to the soil than do prairie roots below a certain depth.

*4.3 What do these differences in inputs tell us about the historical belowground ecosystem under which these soils developed in comparison to the systems under which these soils continue to change?*

The experimental location was a site of cultivation under annual crops for over 100 years. During this time, the soil C profile shifted from having an exponential decrease in C with distance from the surface to a more uniform distribution of C with the highest point of C 10 cm below the surface. Although we do not have values for pre-cultivation soil C, other data from undisturbed sites around the area show that a substantial amount of C has been lost above 30 cm. This loss of C in the soil surface is well known and attributed to mass loss through soil erosion, increased mineralization of organic matter through tillage, and decreased belowground organic matter inputs. However, less is documented about the change in soil carbon below 30 cm.

Discussion Outline

Missing: contribution of exudates, black carbon, DOC, root “pool” is mostly alive, these plots are actually tiled (not very “native”), residue removed

Relationship between prairie roots and the soil C profile

The prairie root C pool establishes more quickly at shallower depths as root systems infiltrate the soil profile over several years. Eventually the whole profile would reach a steady state. Tile drainage may affect rooting depth.

Prairie root C:N ratios increased with depth and over time, especially when not fertilized.

Prairies create a root C pool which can be expected to be larger in soils with less N, given equivalent precipitation.

Contrasts between current annual systems and historical perennial systems

Maize roots have lower C:N ratios that do increase with depth, but not with time.

Maize root C pools are much smaller than prairie root C pools.

Maize root C pools decompose twice as fast as prairie root C pools (0-30 cm).

Maize roots only accumulate at deeper depths.

Planting of prairie does not change soil C profile after 6 years.

Speculation on current contributions to the soil C profile vs. developmental contributions

Maize has lower C:N, which may lead to more stabilized soil C in some soils. Prairie has higher C:N, which may have led to less retainable C. Evidence of increased soil C at depth (Veenstra).

Changes in environment more important than changes in inputs? Soil temp (tillage, residue removal, stand count), soil moisture (subsurface drainage), and soil N (maybe, artificial fertilizer).

Conclusion

C:N ratio change with depth may have been very important in the development of the soil C profile, but I’m not sure what it means. Slower to decompose (important for modeling). Remember, less of the root and more of the C found at depth, proportionally. Texture?

Current systems contribute fewer root inputs, which decompose more quickly, but may be more likely to be stabilized (if the soil is not saturated).

Environmental changes may have a larger effect than differences in inputs near the surface and at depth. (Really? The differences in mass are pretty big.)

C:N had two major implications: 1) Less root contribution and more rhizodeposition contribution in the formation of the soil C profile and 2) Corn contributes more C to the soil than prairie below a certain depth.

|trt |place | splitC| splitR| totalrootC| totalcarbon| proprootC| propcarbon|

|:---|:------|------:|------:|----------:|-----------:|---------:|----------:|

|CC |bottom | 89.30| 0.44| 0.71| 160.46| 0.62| 0.56|

|CC |top | 71.17| 0.27| 0.71| 160.46| 0.38| 0.44|

|P |bottom | 83.40| 1.21| 4.37| 162.54| 0.28| 0.51|

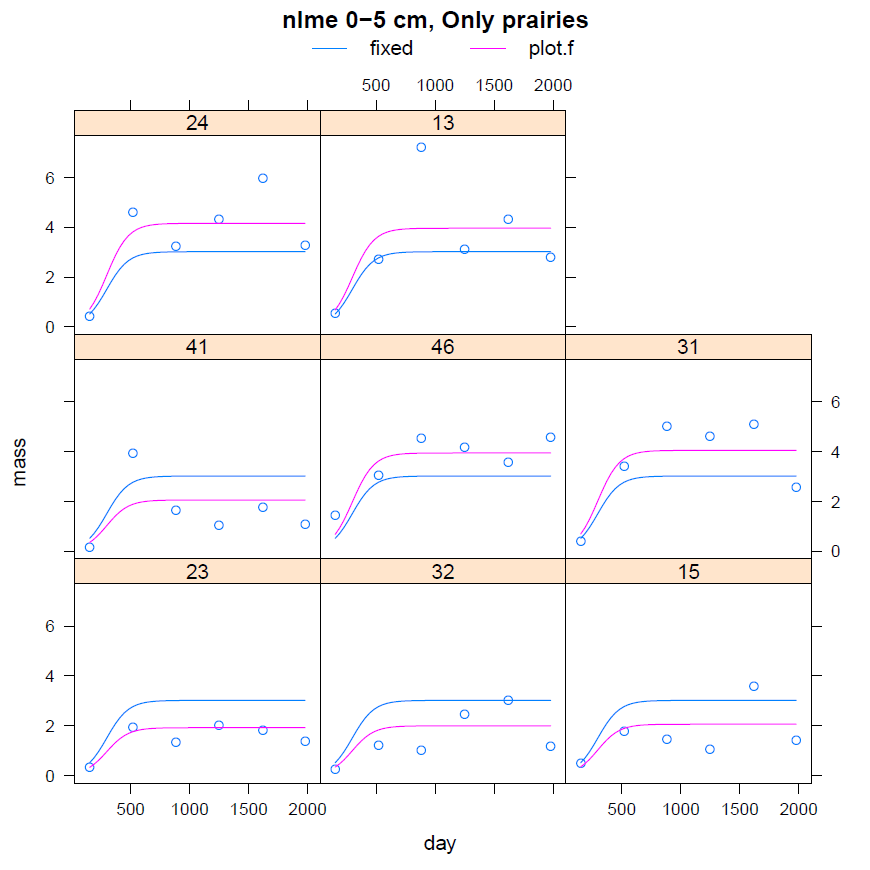
|P |top | 79.14| 3.16| 4.37| 162.54| 0.72| 0.49|

|PF |bottom | 106.96| 0.88| 2.35| 183.63| 0.37| 0.58|

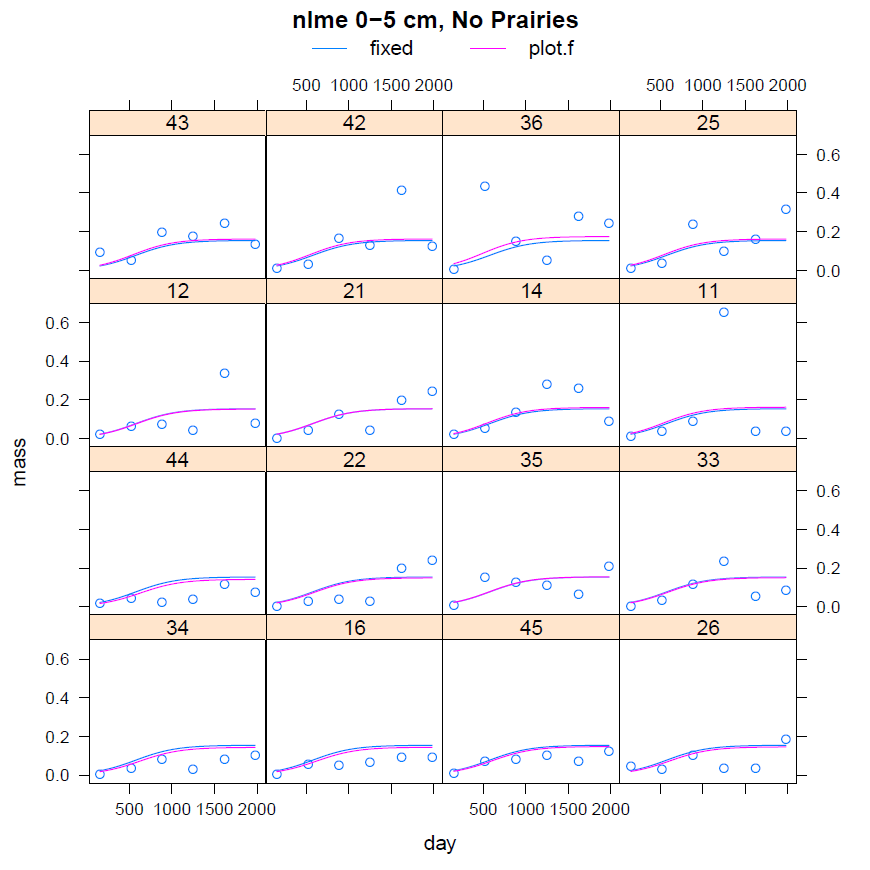
|PF |top | 76.67| 1.47| 2.35| 183.63| 0.63| 0.42|

6. Appendix

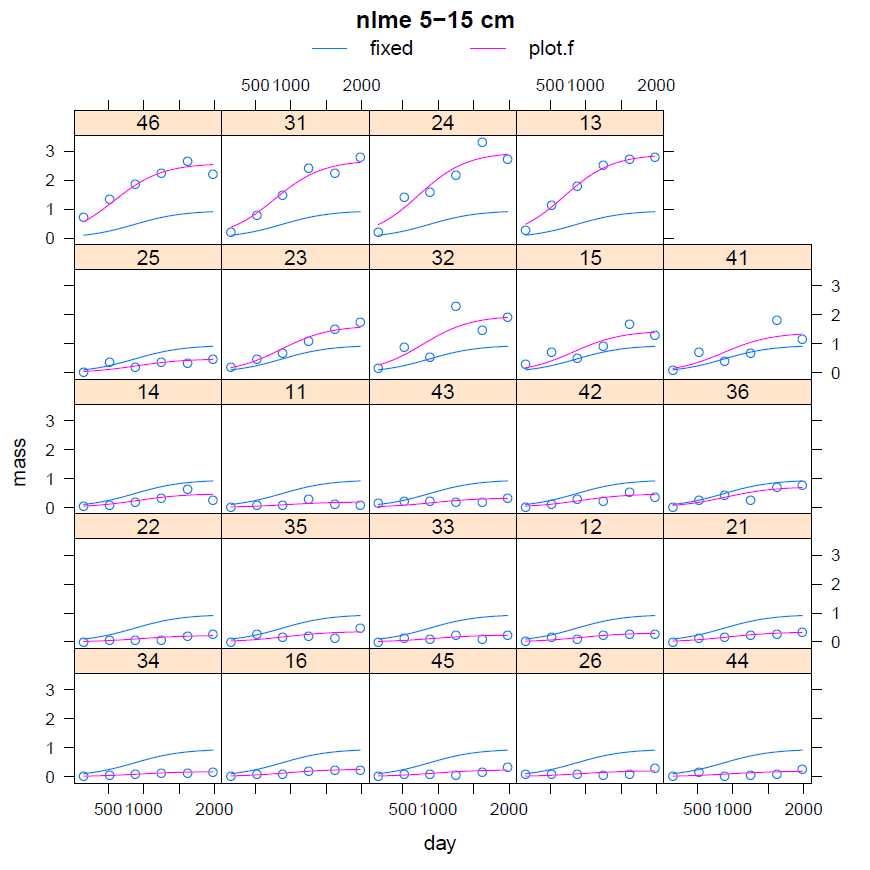
*Logistic curve fits used to generate predicted root accumulation for each depth. Each panel represents one experimental plot (number is plot number).*



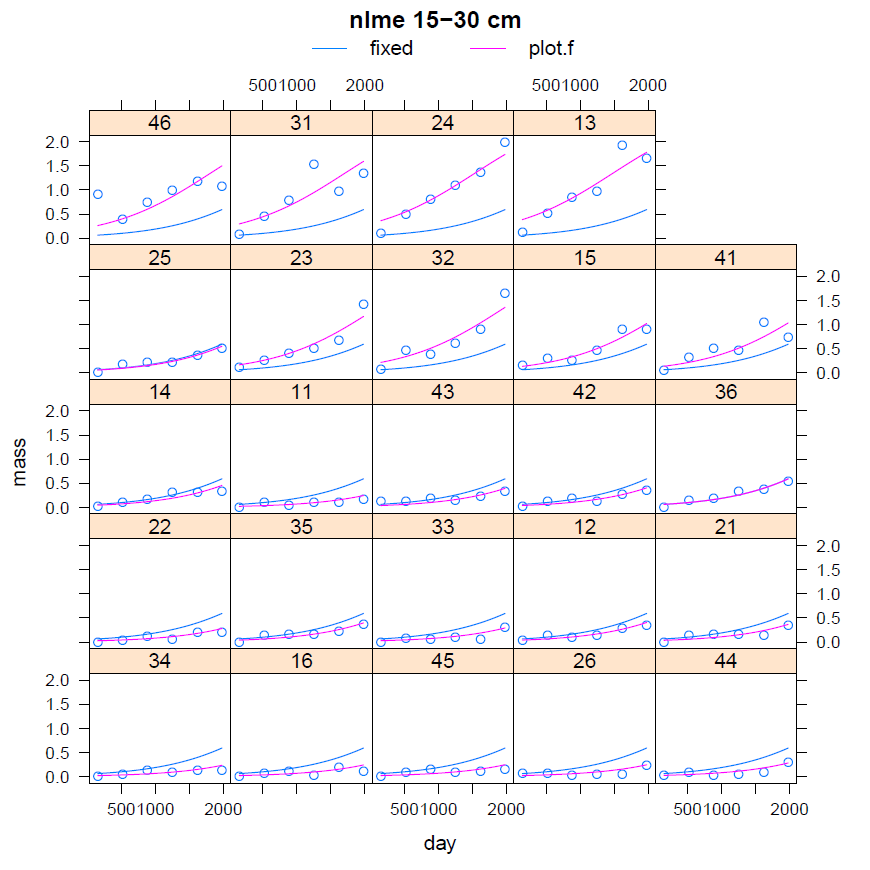
Appendix Fig 1. Fits of logistic curves to 0-5 cm prairie and unfertilized prairie root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.



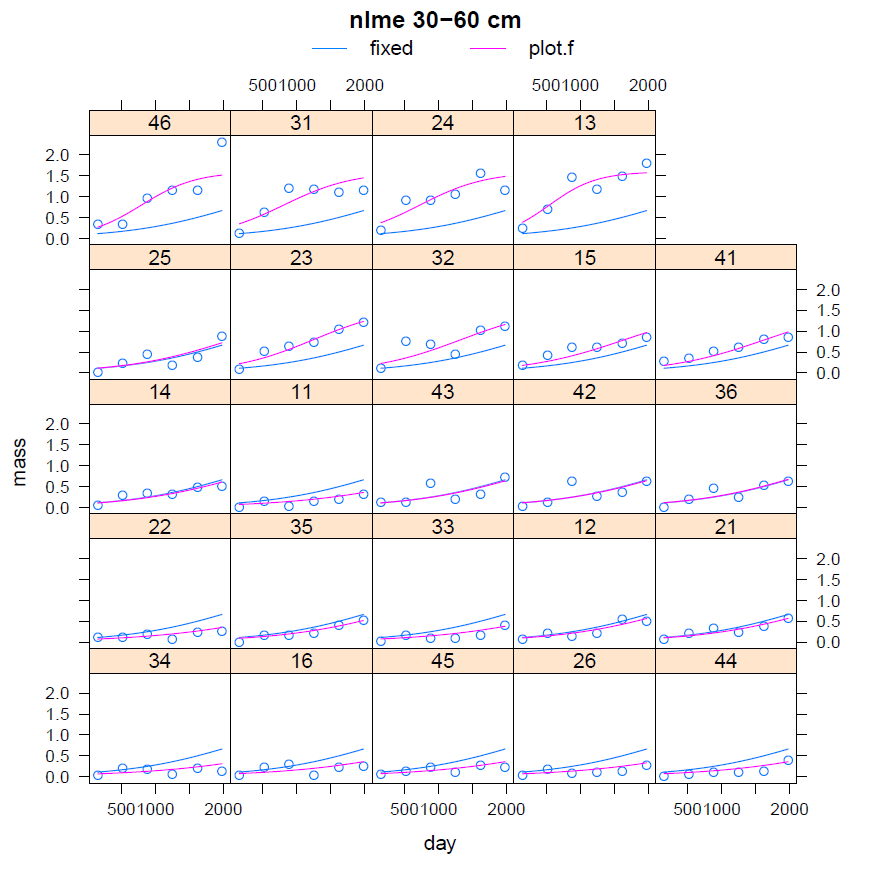
Appendix Fig 2. Fits of logistic curves to 0-5 cm row-crop root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.



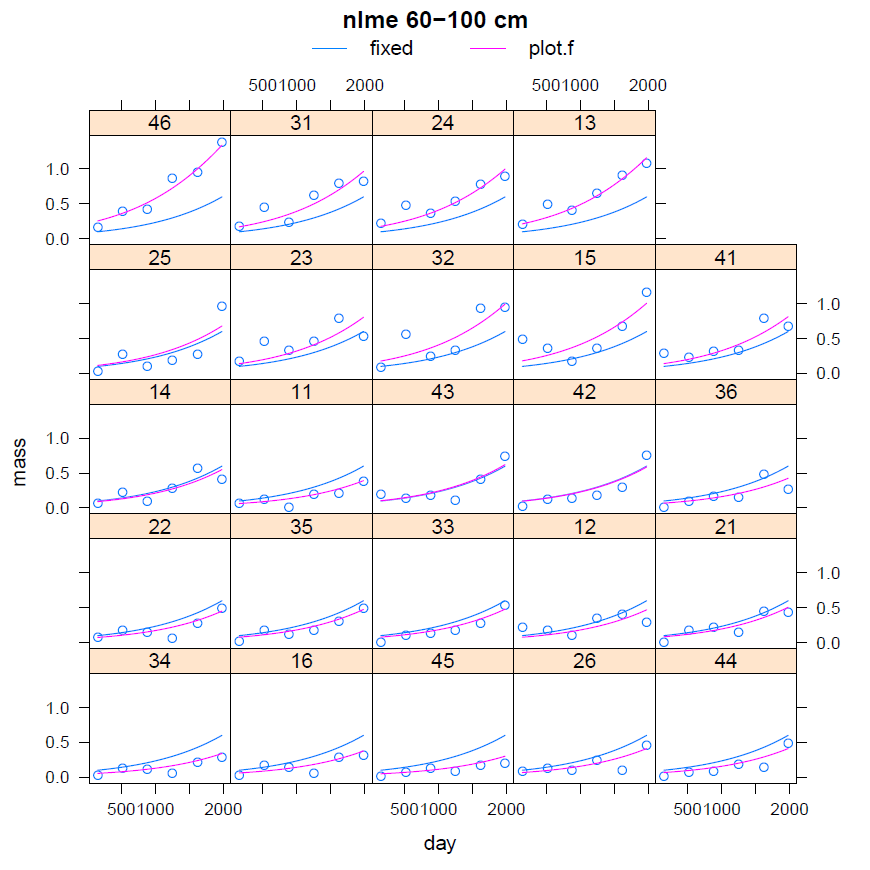
Appendix Fig 3. Fits of logistic curves to 5-15 cm root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.



Appendix Fig 4. Fits of logistic curves to 15-30 cm root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.



Appendix Fig 5. Fits of logistic curves to 30-60 cm root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.



Appendix Fig 6. Fits of logistic curves to 60-100 cm root mass over six years (represented in days after establishment). Pink lines are the fit for each experimental plot and were used to make predictions.