



## Research paper

# Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA



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## ABSTRACT

Fine roots play a key role in the global carbon (C) cycle because much of the C accumulating in soil is the result of fine root production and turnover. Here we explore the effect of plant community composition and diversity on fine root production in surface soils and plant biomass allocation to fine roots in six perennial cropping systems differing in composition and diversity planted as biofuel feedstocks. The six systems were established in 2008 at both a high and a moderate fertility site located in the upper Midwest, USA and included: switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus × giganteus*), hybrid poplar (*Populus nigra* × *P. maximowiczii* 'NM6'), native grasses (a five-species assemblage of *Andropogon gerardii*, *Elymus canadensis*, *P. virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans*), an early successional system, and a restored prairie with 25 sown grass, legume, and forb species. For three years (2011–2013) at both sites ingrowth cores were deployed each spring; half were extracted at mid-season and the rest in late fall. Native grasses and restored prairie systems produced 31–77% more fine roots by mid-season compared to the other cropping systems at both sites. Miscanthus and hybrid poplars tended to have the lowest fine root production. The polyculture cropping systems allocated 39–94% more energy to the production of fine roots, with the exception of switchgrass at the low fertility site. Findings demonstrate a greater potential for diverse biofuel cropping systems to allocate C belowground to fine roots as compared to monocultures, with potential implications for soil C sequestration.

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## 1. Introduction

Fine roots represent ~33% of global net primary productivity [1] and play a key role in the global carbon (C) cycle because the majority of C accumulating in the soil is the result of fine root production and turnover [2]. Fine roots turn over at least once per year, a frequency that has a direct effect on soil C cycling since a portion of the C from senesced roots is incorporated into soil organic matter

[3–5]: as roots senesce, C enters the soil organic matter pool, which globally holds twice the amount of C as the atmosphere [6]. Across different ecosystems fine root turnover can account for 30–80% of organic C inputs into soil [7], and C derived from roots persists longer in soil compared to C derived from aboveground residue [5,8].

It is thus important to better understand fine root production and how it influences C sequestration [9] and to determine strategies that might promote root production and C accrual in managed ecosystems [10,11]. One proposed strategy is to increase crop diversity. The benefits of biodiversity for aboveground production are well known and have been demonstrated in a variety of

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natural [12–14] and managed [15,16] ecosystems. Several studies have hypothesized that biodiversity could also have a positive effect on belowground production. Hooper and Vitousek [13], for example, suggest that root production should be greater in more diverse cropping systems as a result of plant complementarity, in particular as a result of differences in phenology and nutrient demand. De Kroon et al. [17] hypothesized that pathogens constrain root growth in monocultures such that root growth is enhanced in mixed species communities.

Empirical evidence, however, is scant and often conflicting. For example, Fornara and Tilman [18] found that high diversity grasslands on sandy soils in the northern U.S. stored five times more C than did monoculture communities because of greater belowground net primary productivity (BNPP), standing root biomass, and more roots below 60 cm. Bessler et al. [19], on the other hand, found that belowground biomass and root production remained the same across a species richness gradient in a long-term grassland experiment in Europe. Increased fine root production has also been documented in more diverse forest communities in some cases; for example, Liu et al. [20] found mixed forest stands had greater standing fine root biomass and production compared to monoculture stands, although others [21,22] have found no mixed species effect.

Here we used the ingrowth core method to explore patterns of fine root production across six perennial biofuel cropping systems that vary in species composition and diversity. More specifically, we tested the hypothesis that fine root production is greater in diverse cropping systems than in monocultures. Because fine root production can be influenced by soil fertility and precipitation [23], we also contrasted fine root responses in two different locations: on a moderately fertile Alfisol soil in southwest Michigan and on a highly fertile Mollisol soil in south central Wisconsin. We tested responses across three growing seasons, which included a drought year with growing season precipitation significantly lower than the historic average.

## 2. Methods

### 2.1. Site description

This study was conducted at the Great Lakes Bioenergy Research Center's Biofuel Cropping System Experiment (BCSE) located at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research site in southwest Michigan (42°24'N, 85°24'W) and the Arlington Agricultural Research (ARL) station in south central Wisconsin (43°18'N, 89°21'W). Mean annual precipitation and temperature are 1005 mm and 10.1 °C at KBS [24] and 833 mm and 7.4 °C at ARL [25]. Soils at KBS are Alfisols, moderately productive, fine-loamy mixed, semiactive, mesic Typic Hapludalfs of comingled Kalamazoo and Oshtemo series [24]; horizons are Ap (0–30 cm), E (30–41 cm), Bt1 (41–69 cm), 2 Bt2 (69–88 cm), and 2E/Bt (88–152). Surface (0–10 cm) soil pH is 6.1, total soil C is 12.5 g kg<sup>-1</sup> [25], and gravel-free particle size density is 63% sand, 31% silt, and 6% clay [25]. Soils at ARL are Mollisols, highly fertile, silty loam, mesic Typic Argiudolls in the Plano series [26]; horizons are Ap (0–23 cm), A (23–36 cm), Bt1 (36–48 cm), Bt2 (48–79 cm), and Bt3 (79–109 cm). Surface (0–10 cm) soil pH is 6.6, total soil C is 22.0 g kg<sup>-1</sup> [25], and gravel-free particle size density is 9% sand, 66% silt, and 25% clay [25]. Prior to 2008, both sites were under annual row crops for decades.

### 2.2. Experimental design and systems

The BCSE was established in spring 2008 as a randomized complete block experiment with five replicate blocks at each site.

Treatments include annual row crops, monoculture perennial grasses, and diverse perennial grasses and forbs. In this study we sampled from the perennial cropping systems, which include monocultures of switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus × giganteus*), and hybrid poplars (*Populus nigra* × *P. maximowiczii* 'NM6'), and three diverse systems including a native grass assemblage of five species (*Andropogon gerardii*, *Elymus canadensis*, *P. virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans*), an early successional system that represents the seed bank and natural colonization since establishment at the beginning of the experiment, and a restored prairie system planted with 18 different native C3, C4, and legume species. Switchgrass was planted into both the five species native grass and the restored prairie systems at lower densities than the monoculture switchgrass system to prevent switchgrass dominance in the mixed species systems [27]. The monoculture switchgrass system consists of the Cave-in-Rock variety, while the switchgrass in the restored prairie and native grasses consists of the Southlow variety. The early successional community relies solely on volunteer plant growth from pre-existing seed banks and colonizing propagules. Dominant species in the early successional system during this study at KBS included *Conzya canadensis* and *Setaria faberi*; and at ARL, *Lactuca serriola*, and *E. canadensis*. Dominant species in the restored prairie during this study at KBS included *E. canadensis*, *S. nutans*, *A. gerardii*; and at ARL included *E. canadensis*, *Ratibida pinnata*, *Monarda fistulosa*, and *Symphyotrichum novae-angliae*. The Shannon-Weiner diversity index for each system is presented in Table 1. Species names and abundances for all systems are available at [28].

Plots were 27 × 43 m (0.12 ha), separated by 15-m wide mowed alleys planted in turfgrass. Field preparations in the spring of 2008 included chisel plowing and secondary tillage [25]. The switchgrass, native grasses, and restored prairie systems were planted in summer 2008 with a brillion-type native plant seeder. The seeding rate for switchgrass was 7.5 kg ha<sup>-1</sup>. Individual planting densities for the native grasses ranged from 1.6 to 2.4 kg ha<sup>-1</sup> and restored prairie planting densities ranged from 0.4 to 1.2 kg ha<sup>-1</sup>. The individual species seeded weights by hectare was 11.2 kg ha<sup>-1</sup> for both native grasses and restored prairie. Pure live seeding rates were used for all grasses. Both the miscanthus rhizomes and poplar cuttings (25 cm length) were planted in rows by hand in May 2008 at 17,200 ha<sup>-1</sup> and 2778 ha<sup>-1</sup>, respectively. Miscanthus failed at ARL due to winterkill in 2008–2009 [25] and was replanted in spring 2010. Fertilizer nitrogen (N; 56 kg ha<sup>-1</sup>) was applied as ammonium nitrate to switchgrass, miscanthus, and early successional systems each June beginning in 2009 and in 2010 to native grasses. The poplars received a single application of ammonium nitrate fertilizer in 2010 at an N rate of 155 kg ha<sup>-1</sup> at KBS and 210 kg ha<sup>-1</sup> at ARL. The restored prairie system was unfertilized. Weeds were controlled with a broadleaf herbicide application pre- and post-

**Table 1**

Shannon-Weiner diversity index (*H'*) for native grasses, early successional, and restored prairie systems at KBS and ARL for years 2011 (3rd year), 2012 (4th year), and 2013 (5th year).

| Location                  | System             | Year |      |      |
|---------------------------|--------------------|------|------|------|
|                           |                    | 2011 | 2012 | 2013 |
|                           |                    |      | H'   |      |
| High Fertility<br>ARL     | Native Grasses     | 1.09 | 1.34 | 1.23 |
|                           | Early Successional | 1.96 | 1.97 | 1.94 |
|                           | Restored Prairie   | 1.75 | 1.92 | 2.23 |
| Moderate Fertility<br>KBS | Native Grasses     | 1.56 | 1.79 | 1.42 |
|                           | Early Successional | 1.48 | 2.40 | 2.10 |
|                           | Restored Prairie   | 2.04 | 2.24 | 2.20 |

emergence as needed for switchgrass, miscanthus, and native grass in 2010. The miscanthus system received an additional herbicide application in 2011. Harvest for switchgrass, miscanthus, native grasses, early successional, and restored prairie systems occurred in late October at ARL and early November at KBS. Poplars were harvested in December 2013 at ARL and January 2014 at KBS. More details on agronomic practices can be found in Ref. [25].

### 2.3. Fine root production

Ingrowth cores were used to assess the production of fine roots, which we defined as roots <2 mm [1]. To exclude larger roots, cores were constructed of stiff plastic mesh with a hole size of 2-mm. The mesh was stapled to form a cylinder 5 cm in diameter × 13 cm long and closed at the bottom with a plastic cap. Cylinders were filled with soil from cores of equivalent cylinder length to create ingrowth cores. At KBS, soil for the ingrowth cores was taken from individual BCSE plots and sieved to 2 mm in the field, after which washed sand was added in a 3:1 soil:sand ratio. The same procedure was used at ARL, except soil was taken from an adjacent fallow plot. Sand was added to replace volume that was lost due to sieving out roots and stones, to reduce the increased bulk density when repacking the ingrowth cores, and to aid root removal at the end of the incubation period [29]. Following filling, ingrowth cores were inserted upright into 5-cm diameter holes to 13-cm depth at KBS and 15-cm depth at ARL.

Stratified random sampling was used for ingrowth core placement: At each of three permanent sampling stations per plot, one core was placed between plants ~1 m and another between plants ~2 m from the sampling station in a randomly chosen cardinal direction. Each year the cardinal direction was reassigned for the next year's cores. Installation of ingrowth cores at both sites typically occurred in mid-to late-April every year (Fig. 1). The ingrowth cores were extracted twice within each growing season: one core was extracted near the end of July or early August, hereafter referred to as mid-season, and the second core was extracted at the following harvest near the end of October, hereafter referred to as late-season.

Following extraction, cores were taken to the laboratory and refrigerated until they were washed free of soil over a 1 mm sieve. Roots remaining on the sieve (both live and dead) were then immediately dried at 60 °C for 48 h in shallow aluminum tins and then weighed following a 1–2 h cool-down period. Fine root production was calculated as total root biomass divided by number of days in the field. To estimate root production between the mid-season and late-season samplings we subtracted mid-season production from late-season production. We report data from years 2011, 2012, and 2012, when the perennial crops were in their 3rd, 4th, and 5th year, respectively.

### 2.4. Aboveground net primary production

Aboveground Net Primary Production (ANPP) for the herbaceous perennial crops was determined from measurements of maximum aboveground biomass as described in Sanford et al. [25]. In brief, ANPP for switchgrass, native grasses, early successional, and restored prairie systems was determined in mid-August when the crops reached physiological maturity. At three pre-determined stations, 2.0 × 0.5-m quadrats were placed in an east-west direction, except for miscanthus, where we used a 1.5 × 0.6-m quadrat to better match planting patterns. Within quadrats, plant biomass was clipped to ground level and dried at 60 °C for a minimum of 48 h. The dry weight was then determined and recorded. For poplars, ten trees at each site (2 trees/replicate plot) were chosen to cover a representation of trees in each diameter size class for that year. The

selected trees were measured for stem diameter (mm) at 15-cm above the soil surface, basal diameter at soil surface (mm), and tree height of the primary stem (m); the trees were harvested and cut into manageable pieces, the pieces were tagged or bagged for tracking, dried at 60 °C until weight was stable, and the dry weight recorded. Separate allometric equations were developed based on the relationship between harvested biomass and the best fit measured metric [30–32]. For both 2011 and 2012, the best predictive model at KBS was the linear relationship between harvested biomass and diameter at 15-cm above the soil surface (mean  $r^2 = 0.93$ ), while at ARL it was primary stem height (mean  $r^2 = 0.89$ ):

$$Y = \alpha + \beta X + \varepsilon$$

where  $Y$  is the measured tree weight and  $X$  is the best fit predictive variable. In winter 2013, the poplar plots at KBS were harvested using a hydraulic cutting shear. Biomass was then chipped into a truck and weighed field moist using truck scales. Sub-samples were collected and oven dried to determine moisture and yields were corrected to 100% DM. Poplar ANPP values in 2013 at ARL were not used because the trees were infected with a leaf spot fungal disease (*Marssonina* spp.).

### 2.5. Fine root BNPP:ANPP index

Since we calculated fine root production on a per day basis, and because there is no equivalent daily measurement for ANPP, we established a fine root BNPP:ANPP index to compare belowground fine root production among the six systems normalized for differences in aboveground production. Mid-season fine root production was used for fine root BNPP.

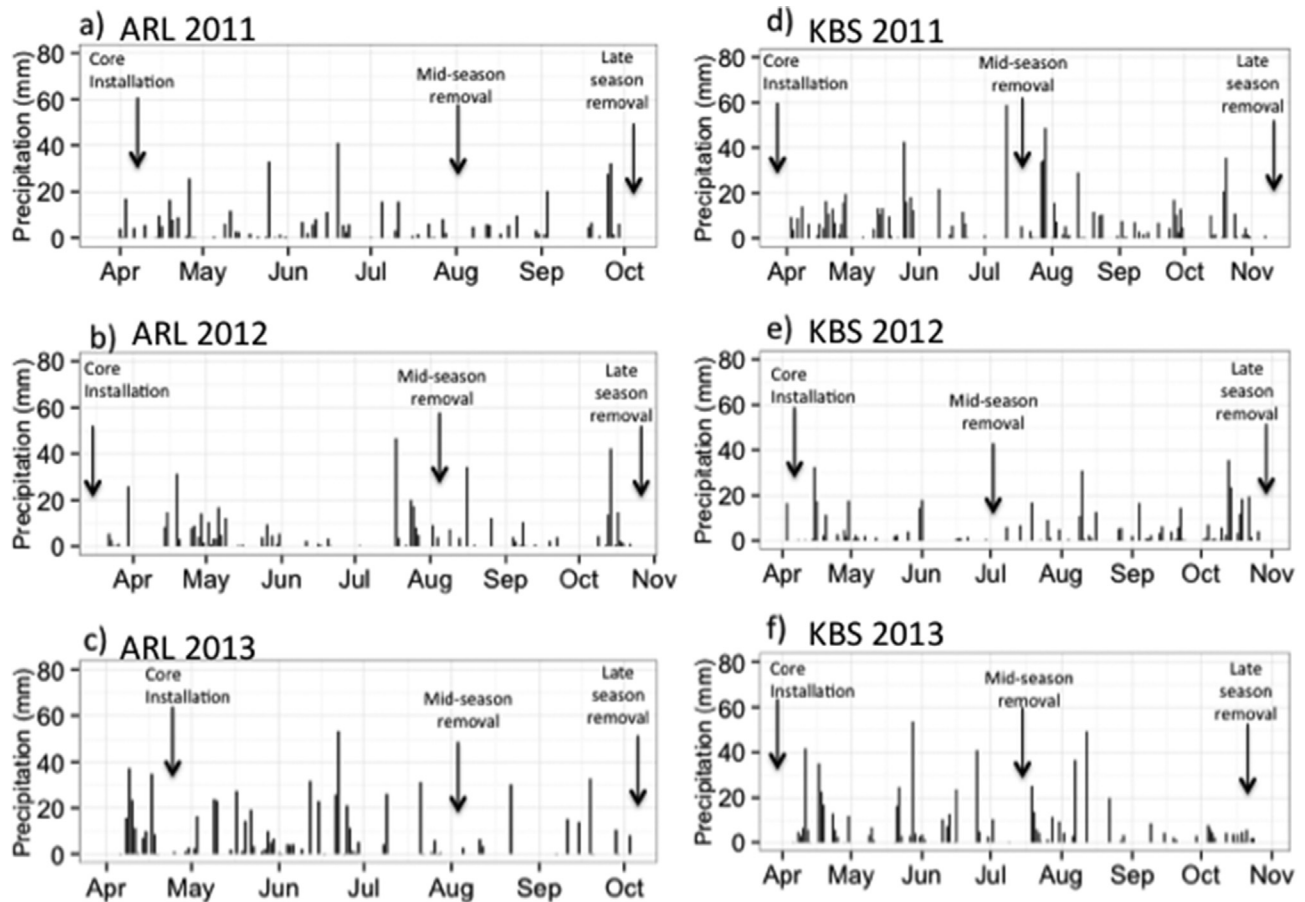
### 2.6. Root depth distribution

The standing stock of live and dead root biomass was determined at the end of the growing season in late November for switchgrass, miscanthus, and poplar. We assessed root biomass by sampling soils with a hydraulic direct-push sampler to a depth of 1 m using a core 7.6 cm in diameter (Geoprobe; Salina, KS at KBS and Giddings probe; Windsor, CO at ARL). Within the switchgrass and miscanthus systems, cores were taken at three locations in each plot: in the direct center of a plant, adjacent to a plant, and in the interstitial space between plants. Three cores were also taken in the poplar system: the first core was taken within 20 cm of a randomly selected poplar tree, the second core was taken mid-way between rows of poplar trees, and the third core was taken half-way between the first two cores. Cores were then divided into four different depth strata: 0–10, 10–25, 25–50, and 50–100 cm. Root mass by depth was determined as for ingrowth cores, above.

### 2.7. Data analysis

Mid-season fine root production and the fine root BNPP:ANPP index were transformed to reduce heterogeneity of variance. We used a square-root transformation and back-transformed after statistical analyses. Thus, geometric means are reported for mid-season biomass and the BNPP:ANPP index. To back transform the standard error, we calculated a 95% confidence interval of the transformed data and then back-transformed the interval [33].

Data were analyzed using Proc Mixed of SAS (version 9.4; SAS Institute, Cary, NC, USA). Cropping system and depth were treated as fixed effects and block as a random effect. There was no site effect because we chose to treat KBS and ARL as separate experiments to avoid pseudoreplication. For mid-season fine root



**Fig. 1.** Timing of ingrowth core installation and precipitation at the high (ARL) and the moderate (KBS) fertility sites during 2011 (3rd year after planting), 2012 (4th year), and 2013 (5th year). Both sets of cores were installed in late March or early April. The first set of cores (mid-season) were removed in mid-July or early August and the second set of cores (late-season) were removed in late October or early November. Arrows indicate when cores were installed and removed. a) ARL 2011, b) ARL 2012, c) ARL 2013, d) KBS 2011, e) KBS 2012, f) KBS 2013.

production, fine root BNPP:ANPP index, and the difference between late- and mid-season production, year was treated as a repeated measure. Significant differences were determined at  $p = 0.05$  and means were compared with an adjusted Tukey's pairwise means comparison.

### 3. Results

#### 3.1. Precipitation

At ARL (the high fertility site), cumulative precipitation during the time the ingrowth cores were installed (April–Oct/Nov) was 451, 491, and 546 mm for 2011, 2012, and 2013, respectively (Fig. 2). ARL always received more rain in the first part of the growing season compared to the later portion (Fig. 2). At KBS (the moderate fertility site), cumulative precipitation was 792, 457, and 616 for 2011, 2012 and 2013, respectively. In both 2011 and 2013, precipitation was above average between April and late October. However, KBS experienced a drought early in the growing season of 2012, with only 152 mm of precipitation by mid-season (Figs. 1 and 2).

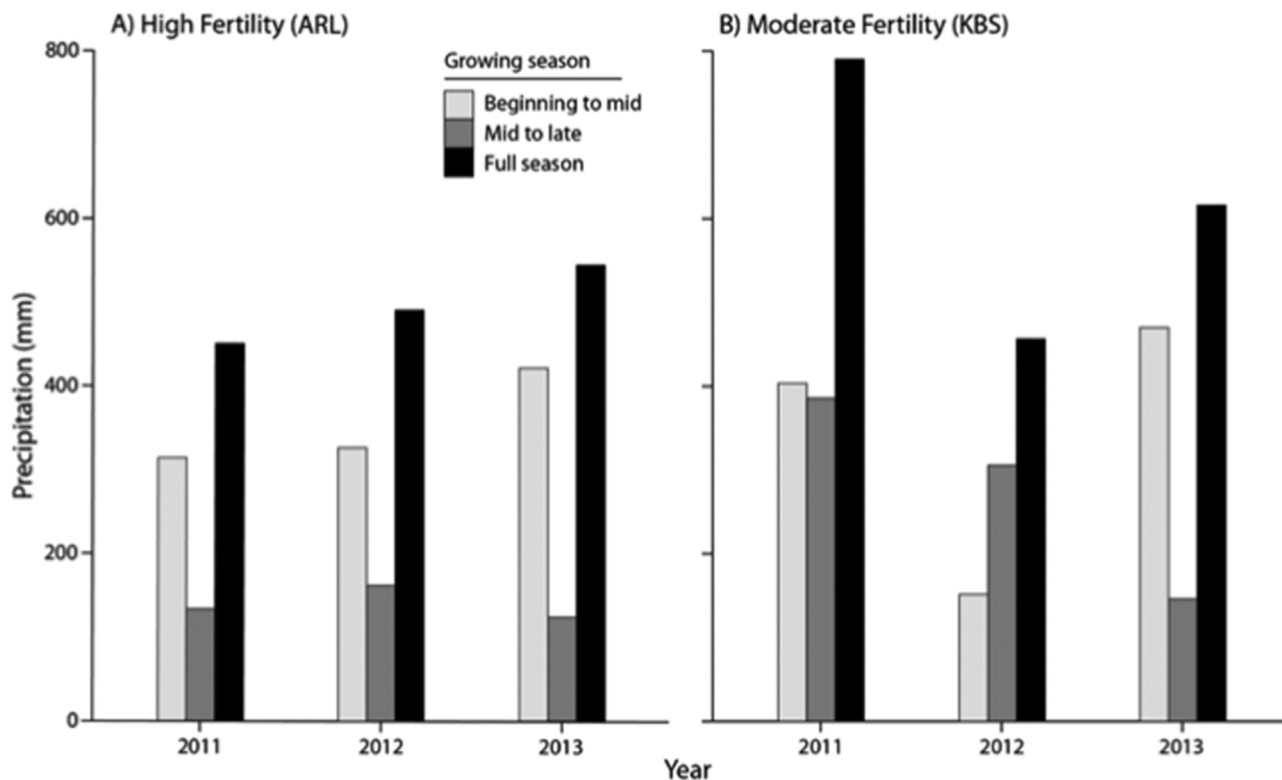
#### 3.2. Fine root production

Mid-season fine root production down to 13 or 15 cm significantly differed across the six cropping systems at both ARL and KBS (Figs. 3 and 4 ARL,  $F = 3.7$ ,  $p = 0.01$ ; KBS,  $F = 4.8$ ,  $P = 0.003$ ). Fine

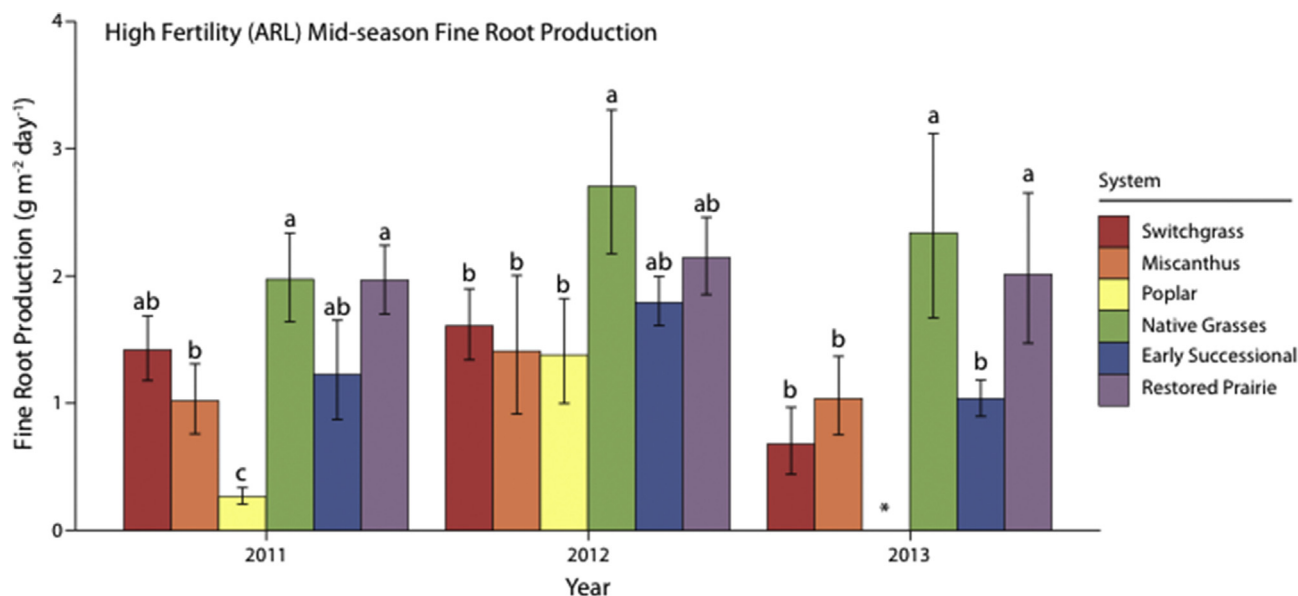
root production also significantly varied from year-to-year (ARL,  $F = 3$ ,  $p = 0.009$ ; KBS,  $F = 12.8$ ,  $p < 0.0001$ ), although trends among the different cropping systems were similar each year.

At ARL, native grasses and restored prairie systems typically had the greatest amount of fine root production (Fig. 3). In 2011, fine root production ranged from  $0.52$  to  $1.40 \text{ g m}^{-2} \text{ day}^{-1}$ , and the native grasses and restored prairie systems had significantly greater fine root production compared to the miscanthus and poplar systems (Fig. 3,  $p < 0.05$ ). In 2012, the native grasses system had significantly greater ( $p < 0.05$ ) fine root production compared to all three monoculture systems with a mean of  $1.65 \text{ g m}^{-2} \text{ day}^{-1}$  for native grasses compared to monoculture switchgrass ( $1.26 \text{ g m}^{-2} \text{ day}^{-1}$ ), miscanthus ( $1.18 \text{ g m}^{-2} \text{ day}^{-1}$ ), and poplar ( $1.18 \text{ g m}^{-2} \text{ day}^{-1}$ , Fig. 3) systems. Similarly, in 2013 the native grasses and restored prairie systems had significantly greater fine root production compared to miscanthus and poplar systems. In 2012 and 2013, production across the monoculture systems was similar. Averaging across years, the native grasses system produced the greatest amount of fine roots ( $2.3 \pm 0.2 \text{ g m}^{-2} \text{ day}^{-1}$ ), while the miscanthus and poplar systems produced the lowest ( $1.2 \pm 0.13 \text{ g m}^{-2} \text{ day}^{-1}$  and  $1.2 \pm 0.5$ ; respectively).

There was a strong year effect at ARL ( $F = 3.0$ ,  $p = 0.0009$ ), which was likely caused by variability in fine root production among the monoculture perennials; fine root production in the diverse systems was relatively consistent across the three years (Fig. 3). For example, the poplar system's fine root production significantly



**Fig. 2.** Precipitation during the two intervals of the growing season for which ingrowth cores were installed. Beginning to mid-season covers the period mid-April to mid-growing season. The mid-to late-season interval covers the remainder of the growing season.

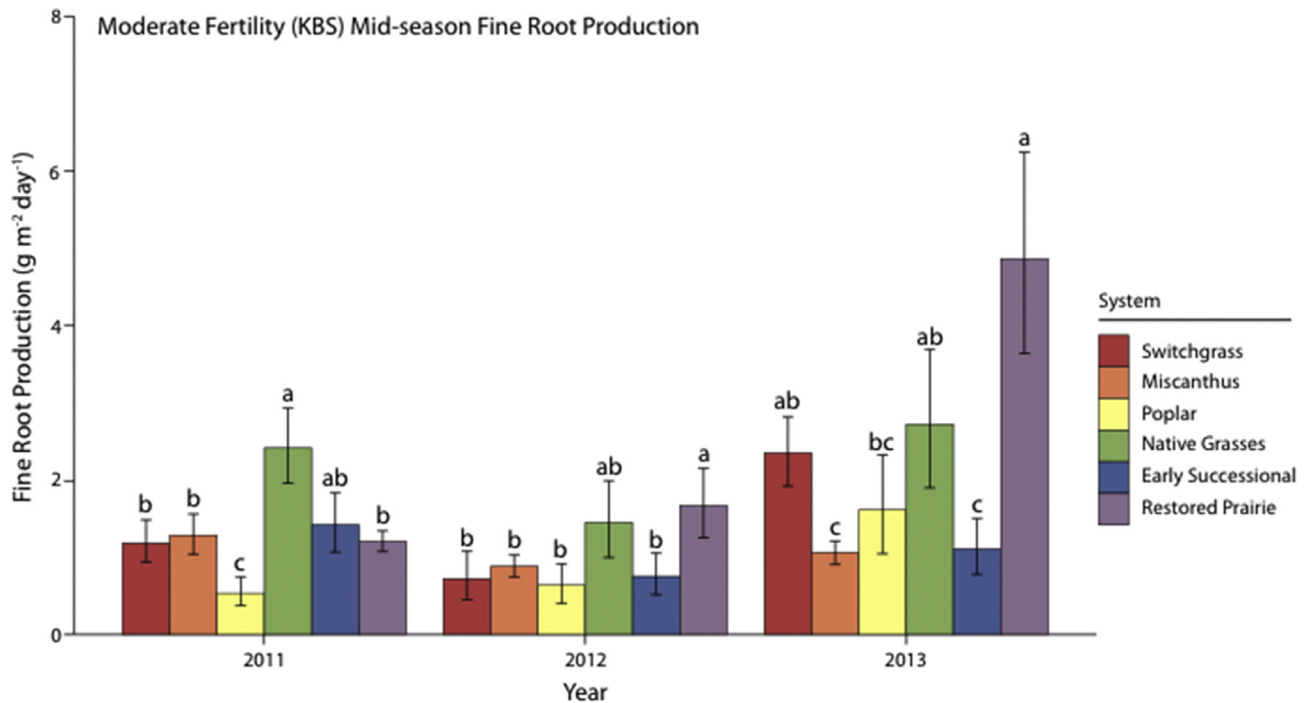


**Fig. 3.** Mid-season fine root production (geometric mean, to 15 cm depth) for six perennial cropping systems at the high fertility site (ARL) in 2011 (3rd year since planting), 2012 (4th year), and 2013 (5th year). Error bars represent back-transformed 95% confidence intervals. Different letters within a given year denote significance at  $\alpha = 0.05$ . \* = Poplar data for 2013 is not available due to a fungal disease outbreak.

increased in 2012 and 2013 ( $p < 0.05$ ), while fine root production in the switchgrass and miscanthus systems tended to decrease over time. Fine root production in the diverse cropping systems stayed remarkably stable over the three years, except for the 2013 early successional system, where production was lower in 2012 by 40%.

At KBS, the native grasses and restored prairie systems also

produced the greatest amounts of fine roots, except in the case of restored prairie in 2011 (Fig. 4). In 2011, the native grasses produced significantly more fine roots than all other systems except for the early successional system. In 2012 and 2013, the restored prairie system had greater fine root production than the other systems except for native grasses and switchgrass systems in 2013. In all



**Fig. 4.** Mid-season fine root production (geometric mean, to 15 cm depth) for six perennial cropping systems at the moderate fertility site (KBS) in 2011 (3rd year), 2012 (4th year), and 2013 (5th year). Error bars represent back-transformed 95% confidence intervals. Different letters within a given year denote significance at  $\alpha = 0.05$ .

years, the poplar and miscanthus systems had the lowest fine root production, except for poplars in 2013. Averaging across years, the restored prairie system produced the greatest amount of fine roots ( $2.6 \pm 1.2 \text{ g m}^{-2} \text{ day}^{-1}$ ) followed by native grasses ( $2.2 \pm 0.4 \text{ g m}^{-2} \text{ day}^{-1}$ ), while the poplar system produced the lowest ( $0.9 \pm 0.3 \text{ g m}^{-2} \text{ day}^{-1}$ ).

In general, fine root production at KBS was greatest in 2013, when production ranged from 1.0 to  $4.8 \text{ g m}^{-2} \text{ day}^{-1}$ , followed by 2011 (Fig. 4). Lowest production occurred in the drought year 2012 when values ranged from 0.8 to  $1.3 \text{ g m}^{-2} \text{ day}^{-1}$ . Differences over time were especially evident for the switchgrass and restored prairie systems, which had significantly greater root production in 2013 compared to prior years ( $p < 0.05$ ).

### 3.3. Fine root BNPP:ANPP index

Fine root BNPP:ANPP differed significantly by cropping system at both sites (Figs. 5 and 6; ARL,  $F = 30$ ,  $p < 0.0001$ ; KBS,  $F = 16.1$ ,  $p < 0.0001$ ). A significant year effect was only evident at ARL ( $F = 72$ ,  $p < 0.001$ ). However, there were significant crop by year interactions at both sites (ARL,  $F = 5.1$ ,  $p < 0.0001$ ; KBS,  $F = 2.1$ ,  $p = 0.05$ ).

Across all years at ARL, fine root BNPP:ANPP in the restored prairie system was significantly greater than in all three monoculture systems (Fig. 5,  $p < 0.05$ ). In 2011, fine root BNPP:ANPP indices ranged from 5.4 to 27.3 for miscanthus and restored prairie systems, respectively. In 2012 and 2013, the diverse perennials always had significantly greater fine root BNPP:ANPP indices compared to the monocultures with the exception of the native grasses (index = 44.5) in 2012, which were not significantly different from the poplars (index = 30.9).

There were also strong temporal trends at ARL. For example, averaging across cropping system, indices in 2012 were 59% greater than in 2011 and 71% greater than in 2013. With the exception of switchgrass and miscanthus systems, all systems' indices were

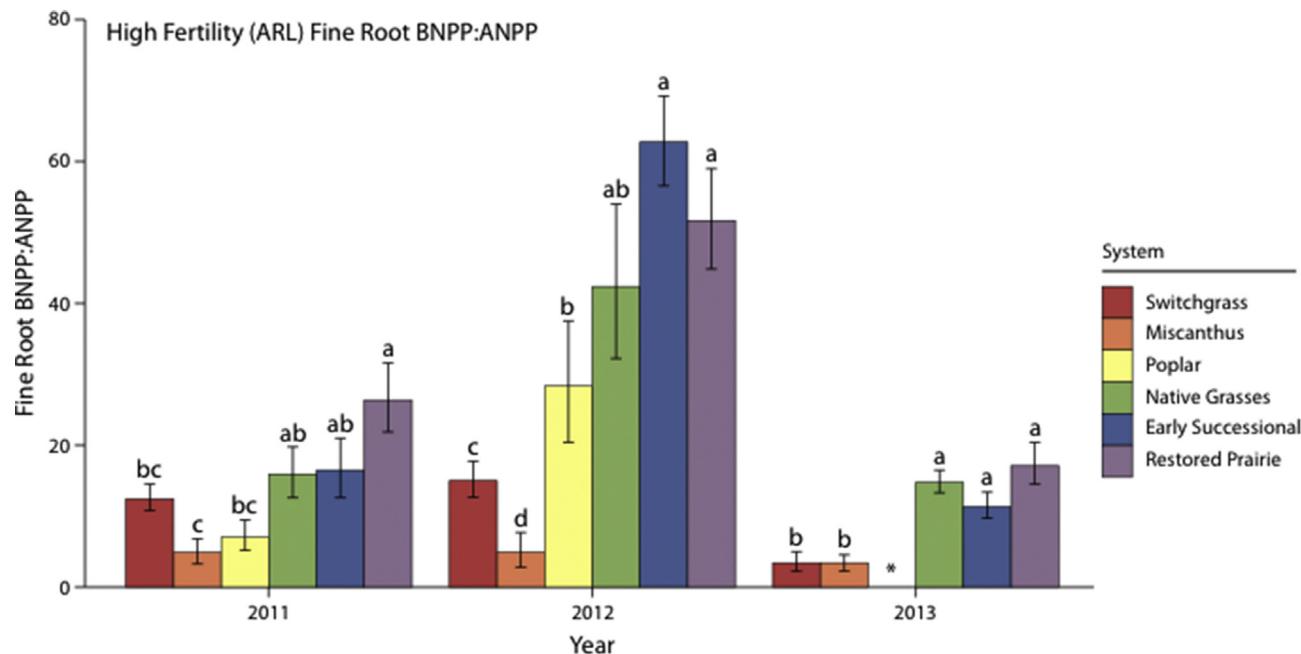
significantly greater in 2012, compared to the other two years ( $p < 0.05$ ).

At KBS, the diverse perennial systems (native grasses, early successional, and restored prairie) always had significantly greater fine root BNPP:ANPP indices than miscanthus and poplar systems, except in 2013, when the early successional system had a lower index (Fig. 6,  $p < 0.05$ ). The switchgrass system had a significantly greater index compared to the other monocultures, except in 2012. In 2011 and 2012, native grasses was the only diverse system that had a significantly greater index than the switchgrass system. Among the diverse perennial systems, there were no significant differences, except in 2013, when the early successional system had a substantially lower index compared to the native grasses and restored prairie systems. Averaging across years, the restored prairie system had the greatest index of  $23.6 \pm 4.3$ , while the miscanthus system had the lowest index of  $4.6 \pm 0.8$ .

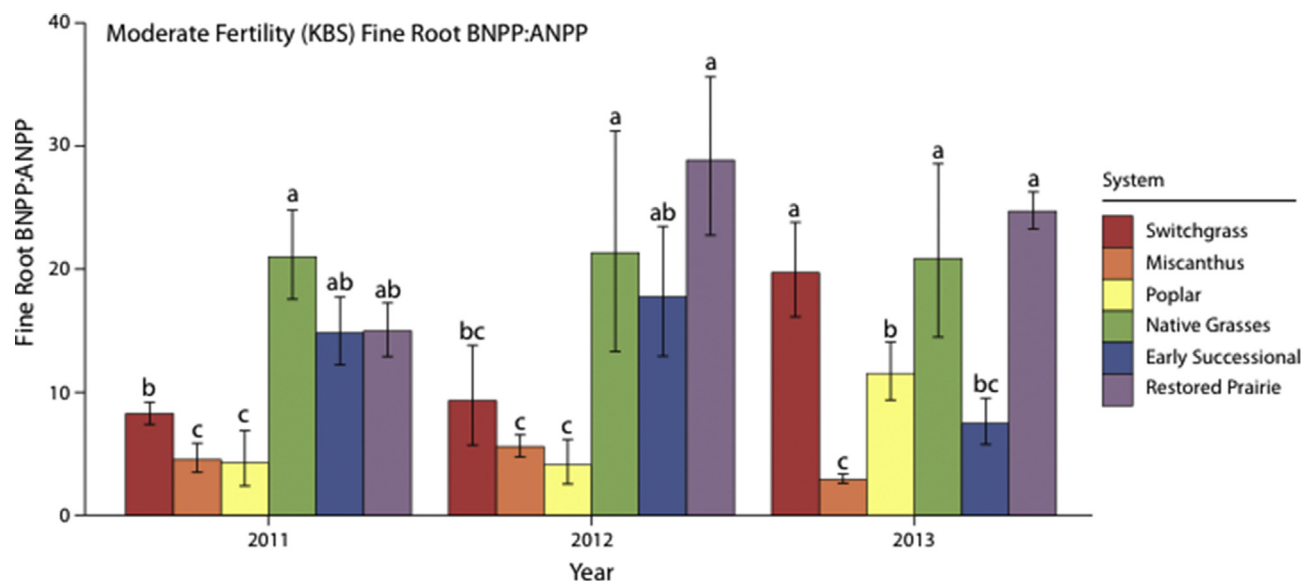
There was no overall year effect at KBS ( $F = 0.3$ ,  $p = 1.3$ ), as fine root BNPP:ANPP remained relatively stable over the three years for all systems. However, there was a significant interaction ( $F = 2.1$ ,  $p = 0.05$ ) due to certain crops with indices that fluctuated through time. For example, pairwise comparisons revealed that the switchgrass system in 2013 had a significantly greater fine root BNPP:ANPP index than in 2011 and 2012 ( $p = 0.02$  and  $0.03$ , respectively).

### 3.4. Late-season vs. mid-season fine root production

We calculated the difference between late-season and mid-season fine root production to reveal the pattern of production in a given growing season. Maximum fine root production did not differ among cropping systems at either site (Table 2, ARL,  $F = 2.0$ ,  $p = 0.1$ ; KBS,  $F = 1.1$ ,  $p = 0.3$ ). However, there were noteworthy differences through time, as the year effect was marginally significant at both sites (ARL,  $F = 2.7$ ; KBS,  $F = 2.8$ ,  $p = 0.07$   $p = 0.08$ ). At ARL, the majority of cropping systems exhibited maximum fine root



**Fig. 5.** Fine root BNPP:ANPP Index (geometric mean) for six perennial cropping systems at the high fertility site (ARL) in 2011 (3rd year), 2012 (4th year), and 2013 (5th year); as described in Methods, the index represents the relative allocation of biomass to fine root production. Error bars represent back-transformed 95% confidence intervals. Different letters within a given year denote significance at  $\alpha = 0.05$ . \* = Poplar data for 2013 is not available due to a fungal disease outbreak.



**Fig. 6.** Fine root BNPP:ANPP Index (geometric mean) of six perennial cropping systems at the moderate fertility site (KBS) in 2011 (3rd year), 2012 (4th year), and 2013 (5th year). Error bars represent back-transformed 95% confidence intervals. Different letters within a given year denote significance at  $\alpha = 0.05$ .

production by the middle of the growing season in both 2011 and 2012. However, in 2013 the switchgrass, miscanthus, native grasses, and restored prairie systems had greater root production in the later part of the growing season. In contrast, maximum fine root production at KBS tended to occur in the later part of the growing season for almost every crop, especially in 2012. Exceptions were the poplar and native grasses in 2011, and the restored prairie system in 2011 and 2013.

### 3.5. Root depth distribution

In general, root biomass was strongly concentrated in the surface for the switchgrass and miscanthus systems (Fig. 7A). At ARL, 77% of total miscanthus root biomass was found in the surface 10 cm, 88% in the top 25 cm, and 94% in the top 50 cm. At KBS, the miscanthus system displayed a similar pattern with 77% of total root biomass in the surface 10 cm, 92% in the top 25 cm, and 96% in

**Table 2**

Difference between end of season fine root production and mid-season fine root production at ARL and KBS for years 2011 (3rd year), 2012 (4th year), and 2013 (5th year). Numbers represent the mean and standard error (in parentheses) for each system. A positive number indicates greater root production in the later part of the growing season and a negative number indicates greater root production during the first part of the growing season.

| Location               | System             | Year Difference |            |            |
|------------------------|--------------------|-----------------|------------|------------|
|                        |                    | 2011            | 2012       | 2013       |
| High Fertility ARL     | Switchgrass        | −0.3 (1.3)      | −0.4 (0.7) | 1.1 (0.9)  |
|                        | Miscanthus         | −0.4 (0.8)      | −0.5 (1.1) | 1.4 (0.9)  |
|                        | Poplar             | −0.3 (0.2)      | −2.0 (0.7) | −3.1 (1.7) |
|                        | Native Grasses     | −2.1 (0.9)      | −1.5 (1.5) | 0.4 (1.1)  |
|                        | Early Successional | −1.8 (0.6)      | −1.3 (0.6) | −0.1 (0.5) |
|                        | Restored Prairie   | −0.5 (0.7)      | −1.0 (0.8) | −0.4 (1.6) |
| Moderate Fertility KBS | Switchgrass        | 0.5 (0.8)       | 1.4 (0.5)  | 0.2 (1.1)  |
|                        | Miscanthus         | 0.6 (0.6)       | 0.8 (0.4)  | 2.3 (0.7)  |
|                        | Poplar             | −0.4 (0.5)      | 1.1 (0.5)  | 0.3 (0.7)  |
|                        | Native Grasses     | −0.02 (0.3)     | 2.2 (0.6)  | 3.0 (1.6)  |
|                        | Early Successional | 1.3 (0.5)       | 0.3 (0.2)  | 0.2 (0.6)  |
|                        | Restored Prairie   | −0.3 (0.4)      | 2.1 (0.4)  | −2.4 (2.1) |

the top 50 cm. At ARL, the root biomass distribution for switchgrass was very similar to that for miscanthus, with 76% of switchgrass root biomass found in the surface 10 cm, 85% in the top 25 cm, and 91% in the top 50 cm. At KBS, switchgrass root biomass was more evenly distributed in the soil profile with 67% of root biomass in the surface 10 cm, 79% in the top 25 cm, and 89% in the top 50 cm. Root biomass in the 50–100 cm depth interval ranged from 4 to 11% for both miscanthus and switchgrass systems at both sites.

Poplar root distributions at KBS (for a nearby site on the same soil series planted at the same time [24]) was more evenly distributed throughout the soil profile (Fig. 7B): 57% of total root biomass was found in the surface 10 cm, 68% in the surface 0–25 cm, and 85% in the surface 0–50 cm.

#### 4. Discussion

At both sites, perennial cropping systems with greater plant diversity produced more fine roots than did monoculture systems, except for the early successional systems where a number of dominant species were annuals rather than perennials. Further, systems with greater diversity consistently allocated a greater proportion of total productivity to fine roots.

##### 4.1. Plant community composition influences mid-season fine root production and allocation

In general, the native grasses and restored prairie systems produced more fine roots than miscanthus, switchgrass, and poplar systems at both sites over all years, while fine root production in the early successional system was always more similar to that in monoculture systems. These findings are broadly similar to those from studies of fine root production in less versus more diverse forests [34,35] and grasslands [18,36]. Furthermore, in a recently published meta-analysis, researchers found that across natural forests, planted grasslands, croplands, and pot systems, species mixtures had 28% more fine root biomass than monoculture systems [37].

Greater fine root production in our native grasses and restored prairie systems may be driven by one or two dominant species. For example, *E. canadensis* (a C3 perennial) was dominant in both of these systems for all years except 2013 at ARL. In general, C3 perennial plants have more fibrous root systems that could

contribute more to fine root production compared to C4 species, which tend to have more coarse roots [38]. Thus, C3 dominance may have contributed disproportionately to greater fine root production in these systems.

Lower than expected fine root production in the early successional systems may also be a species effect in that the early successional system included annual dominants. For example, at KBS, the moderate fertility site, annuals accounted for the majority of biomass in the early successional communities (79%) but in the perennial grass and prairie communities accounted for much smaller percentages (1% and 3%, respectively). Annual dominants included *Conyza canadensis* (31% of total biomass) and *S. faberi* (17%). Annual species like *C. canadensis* typically produce less root biomass than perennials – especially perennial grasses [39]. At ARL, the high fertility site, annuals comprised a small percentage of biomass in the native grasses (6%) and prairie systems (1%), but, at KBS, annuals were abundant in the early successional system (33%).

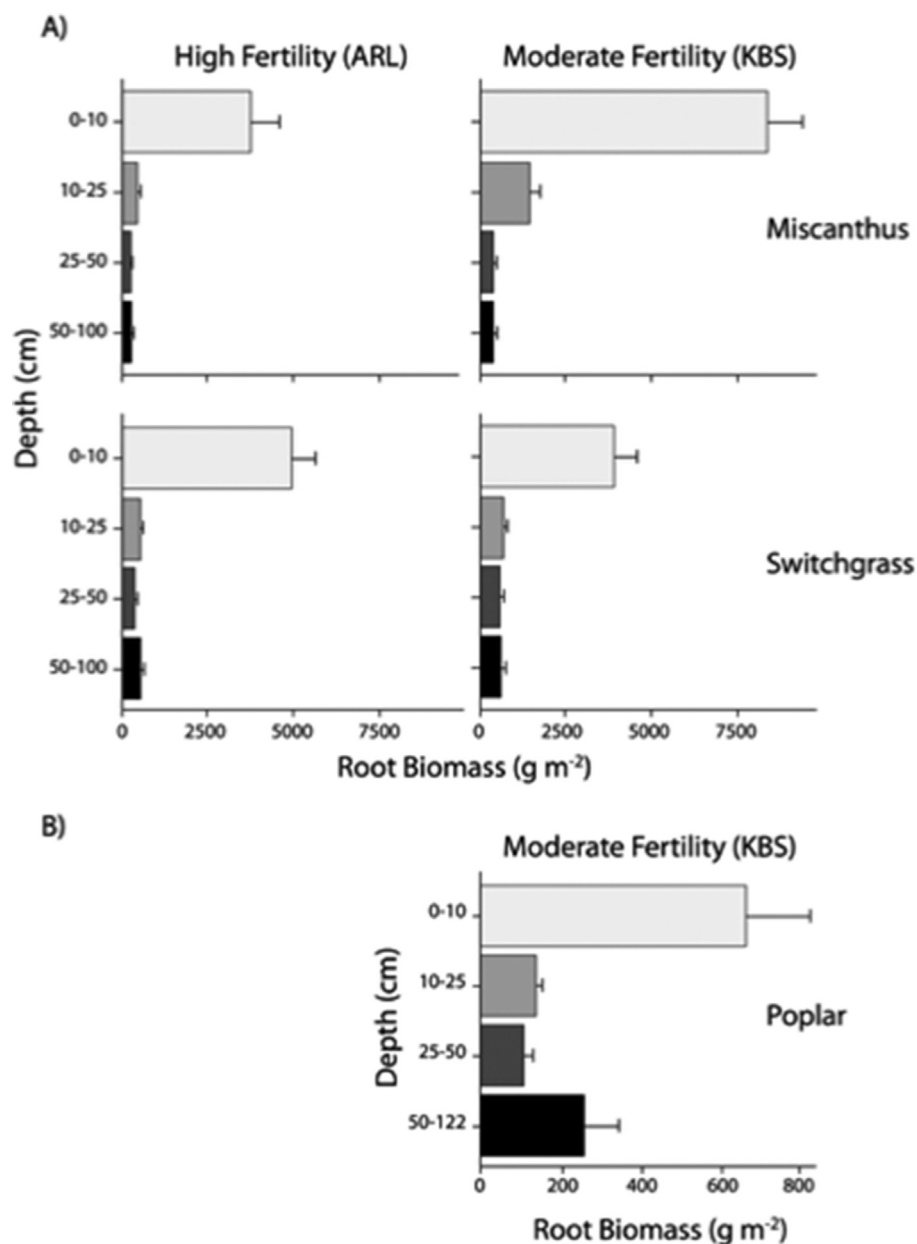
Most authors attribute greater fine root production in systems with higher species diversity to plant complementarity or the diversity-productivity hypothesis [12,13]. In both cases more diverse systems are expected to have greater root production because of greater differences in rooting depths stemming from variation in phenology and plant nutrient demand [12,17,40]. For instance, higher species diversity can lead to species' achieving peak biomass at different times, creating a scenario whereby soil nutrient resources are continuously extracted (i.e., temporal niche partitioning). This, in turn, can lead to increased competition for nutrients and could stimulate greater root production. In addition, the presence of a dominant species can influence temporal niche partitioning in rooting depths that lead to the exclusion of certain species. For example, Fargione and Tilman [41], demonstrated that a dominant C4 bunchgrass excluded other shallow rooted species and instead coexisted with a greater proportion of deep rooted species, leading to overall greater root biomass within the system.

##### 4.2. Belowground allocation

Our fine root BNPP:ANPP index indicates relative investment in belowground vs. aboveground production, and with few exceptions we found that fine root BNPP:ANPP was greater in diverse cropping systems compared to monoculture systems. This suggests that at both sites plants in diverse systems allocated proportionately more biomass to roots than did plants in monoculture systems. This trend contrasts with Bessler et al. [19], who found a decrease in root:shoot ratios with increased diversity at an experiment in Germany. Bessler et al. [19] suggested that the plant complementarity effect led to more available N in the diverse cropping systems, causing a reduction in belowground biomass and greater allocation to aboveground biomass. In our study, plant complementarity likely had the opposite effect, whereby root biomass increased in response to more available N, causing an overall greater investment to fine root biomass production [42]. The greater proportion of legumes in Bessler et al. [19] compared to our study likely explains this disparity. Legumes in our restored prairie systems accounted for ~5% of species composition, while legumes in Bessler et al. [19] accounted for ~20%, likely prompting reduced belowground biomass due to an abundance of N supply as described by optimal partitioning theory [43]. Furthermore, contrasts in species evenness between our two studies likely influenced the timing of fine root production and turnover, which could ultimately lead to differences in biomass allocation [44].

##### 4.3. Timing of maximum fine root production

Based on differences between late- and mid-season fine root



**Fig. 7.** A) Miscanthus and switchgrass root biomass distribution averaged across 2011 (3rd year), 2012 (4th year), and 2013 (5th year) to 1 m at the high (ARL) and moderate (KBS) fertility sites. B) Poplar root biomass distribution to 1.22 m from a nearby site at KBS.

production over the three study years, systems at ARL produced most of their fine roots in the middle of the growing season. This suggests that roots were decomposing and turning over in the later stages of the growing season. Systems at KBS, on the other hand, produced most of their fine roots in the latter half of the growing season. This was especially pronounced in 2012, possibly arising from drought conditions in the early part of the 2012 growing season, which likely slowed root production. Root production would have increased when rainfall resumed in the second part of the growing season, as has been shown for systems elsewhere [45–48]. For other years, contrasting trends between the two sites may reflect soil N availability differences insofar as fine root turnover can be faster in systems with greater N availability [47], which is true for ARL, the site higher in soil fertility. Thus, it seems

plausible that roots persisted longer in the growing season at KBS, where soils have less available N compared to ARL.

Differences between KBS and ARL in the timing of fine root production also demonstrated the need to measure fine root production more than once per growing season. A long-standing view is that maximum belowground production occurs in the middle of the growing season, with greater root decomposition and turnover later in the season [21]. For this reason, most investigators sample root production only once per season [49–51]. This approach would have led to an underestimation of fine root production at KBS, underscoring the need for multiple sampling times [52,53]. Thus, the sequential coring and the maximum-minimum method for quantifying fine root biomass would have helped to capture changes in fine root production throughout the growing season [52,53].

#### 4.4. Root depth distribution

A potential limitation of this study is that our ingrowth cores captured fine root production to a depth of only 13–15 cm, which might affect our conclusions if fine root production at depth interacted by system. However, Bessler et al. [19] found that species richness and diversity did not affect root biomass production at lower depths. Furthermore, similar research at the KBS site measured fine root biomass to a depth of 1 m in an annual and perennial system over a three-year period, and did not detect differences in fine roots in any of the three years, including a drought year [54]. Finally, deep cores from both the KBS and ARL sites indicate that approximately 80% of root biomass is found in the surface 10 cm for at least switchgrass and miscanthus. Thus, it seems reasonable to conclude that the ingrowth cores captured the majority of the fine root production in grass-based systems, and that ingrowth cores to this depth are sufficient to make valid cross-system comparisons. That in the poplar system only ~57% of roots were found in the surface 10 cm is consistent with general patterns of tree roots' being more uniformly distributed with depth than roots of grasses [55]. Nevertheless, the consistently lower fine root production of the poplar system (compared to the other systems except miscanthus) could be an artifact of only sampling the surface 13–15 cm.

#### 4.5. Root patterns of bioenergy cropping systems and potentials for soil C accumulation

It is important to note that we examined fine root production rather than root standing stock. Had we measured total root biomass, we might have found different patterns between the monoculture and diverse perennial cropping systems. For example, miscanthus is known for producing large rhizomes [56] and numerous studies have found that switchgrass produces large and extensive roots to 120 cm [56,57]. In a recent study comparing belowground biomass of monoculture and polycultures of native grasses, Jungers et al. [58] report that switchgrass had significantly greater total standing root biomass compared to the 24 species grass/legume system. In addition, several authors have demonstrated that monoculture perennial cropping systems store large amounts of C in their root systems [59], which has been shown to accumulate soil C overtime [60]. Such findings underscore the contributions that perennial monoculture biofuel systems can make towards soil C storage and overall sustainability compared to annual systems [61].

Notwithstanding patterns of standing root biomass, fine root production should be quantified separately because the process of fine root production and turnover is the primary source of soil C inputs [2,62]. For example, while fine roots often comprise <5% of total biomass, they account for nearly 50% of cycled C in forest, grasslands, and crops [4,63]. Thus, greater fine root production and relative biomass allocation to fine root production within the diverse systems could have important implications for C sequestration.

## 5. Conclusions

Native grasses and restored prairie systems had greater mid-season fine root production than monoculture systems at both the highly fertile ARL site on Mollisol soils and the moderately fertile KBS site on Alfisol soils, suggesting that more diverse systems produce more fine roots irrespective of soil type. Fine root production in the early successional system was more similar to the monoculture systems, probably because dominance by annuals reduced system-level fine root production. Our findings provide

further evidence that plant diversity increases fine root production, which in turn has important implications for soil C accumulation.

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