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# Strategic use of native species on environmental gradients increases diversity and biomass relative to switchgrass monocultures

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ABSTRACT

Switchgrass (*Panicum virgatum*) monocultures are a leading feedstock choice for producing cellulosic biofuels. However, in natural stands, switchgrass is only dominant in a narrow ecological niche of the Tallgrass Prairie. This suggests that strategically selected monocultures or binary mixtures of species, adapted to particular ecological niches, might outyield switchgrass monocultures while increasing biodiversity at the field and landscape scales. To test this hypothesis, we planted monocultures of switchgrass and three alternative species at each of three landscape positions (shoulderslope, midslope, and footslope). Alternative species were also mixed with switchgrass such that they composed 33 or 67% of the total number of plants in each plot. Alternative species at each position included a $C_3$ grass, a $C_4$ grass, and a forb. Biomass data were collected in autumn during each of the two consecutive years following the establishment year. At the shoulderslope, the highest-yielding treatments were those containing little bluestem (*Schizachyrium scoparium*; 12.0 Mg ha$^{-1}$) and those dominated by switchgrass (10.4 Mg ha$^{-1}$). At the midslope and footslope, there were interactions of year × treatment ($p < 0.05$). In general, the highest-yielding treatments at the midslope were those containing big bluestem (*Andropogon gerardii*; 16.4 Mg ha$^{-1}$). At the footslope in 2013, the prairie cordgrass (*Spartina pectinata*) monoculture and treatments with at least 67% of the plants being cup plant (*Silphium perfoliatum*) produced the most biomass (24.3 Mg ha$^{-1}$; $p < 0.05$) but there were few yield differences at the footslope in 2014. This research demonstrated that biomass yield of two- and three-year-old stands of monocultures or binary mixtures of native plant species with switchgrass, when appropriately matched to their natural landscape positions, produced biomass in equal or greater amounts than switchgrass monocultures.



## 1. Introduction

Ecologists have long recognized that native plant communities are comprised of species with similar environmental tolerances (Curtis, 1959; Daubenmire, 1974; Weaver and Albertson, 1956). Yet, each species in a community has a unique niche; no two species respond identically to environment in both time and in space (Hutchinson, 1965). Whittaker (1970) found that the ecological relationships among species across heterogeneous landscapes can be elucidated by plotting the presence and abundance of each species along environmental gradients, particularly those of soils (especially soil moisture), aspect, and disturbance regime. Using this analytical approach, termed direct gradient analysis, Whittaker confirmed that the realized niche space was unique for each species and that plant species varied in their niche breadth, abundance, and extent of niche overlap with neighboring species along these gradients. Similar studies of strong environmental gradients in Dakota prairies that ranged from wetlands up to dry grassland found that some species were adapted to the wet end of the gradient, others the dry end, and yet others the mesic position (Dix and Smeins, 1967; Johnson et al., 1987). Species turnover along the slope was high, and no species occupied an entire gradient. Clearly, development of natural ecosystems has generally produced more numerous, narrowly-adapted species that compete for resources less with each other than a single or a few species

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dominating entire landscapes, except perhaps in extreme environments such as desert, tundra, or chronically-disturbed landscapes dominated by ruderal species.

In contrast to the diversity found in natural plant communities, growing a single species generally reduces management effort. However, agricultural fields are rarely homogeneous with regard to soils and topography; consequently, some areas are more suited and productive than others for the selected monoculture crop. A common response is to alter the environment to suit the crop by, for example, applying nutrients to infertile soils or draining shallow wetlands.

Producing biofuels using annual crop monocultures provides fewer ecosystem services than more diverse plant communities dominated by perennial species. For instance, perennial-based systems can reduce soil erosion, provide wildlife habitat, regulate water and nutrient cycles, and provide refugia for natural enemies of crop pests (Schulte et al., 2006; Sanderson et al., 2013). Diversification of annual and perennial systems can further enhance provision of certain services, including ecosystem resilience and pest suppression (Myers, 1996; Altieri, 1999).

Forage crops have been targeted as potential sources of biofuel feedstock (Sanderson and Adler, 2008), which generated interest in increasing biomass yield and energy conversion technologies. A leading candidate to provide biofuel feedstock is switchgrass (*Panicum virgatum*), a perennial, $C_4$ grass native to the North American Great Plains. It is adapted to a wide range of climates, extending from southern Canada to northern Mexico (Casler, 2002) and has the potential to produce large quantities of biomass with relatively few external inputs, such as fertilizer and herbicide. Its low input requirements are largely responsible for switchgrass having a much better energy efficiency ratio than corn (*Zea mays*) grain ethanol (Hill et al., 2006; Schmer et al., 2008).

Although switchgrass produces high yields across a wide range of environments and topographic positions, it did not dominate the historic Tallgrass Prairie landscape—switchgrass was typically found within a narrow topographic band at the lowland-upland interface, while other plants dominated downslope (e.g., prairie cordgrass [*Spartina pectinata*]) or upslope (e.g., big bluestem [*Andropogon gerardii*] and little bluestem [*Schizachyrium scoparium*] (Weaver, 1954; Dix and Smeins, 1967; Johnson et al., 1987)). This suggests that better-adapted species might outyield switchgrass, especially at the margins of switchgrass's topographic range. Thus, increasing the number of species used for biofuel plantings might increase yields, in addition to addressing concerns that biofuel monocultures will replace diverse natural lands (Dauber et al., 2010; Firbank, 2008).

“Sculptured seeding” (Jacobson et al., 1994) is a revegetation technique (Inlow, 2010; Wark et al., 2015) that adopts elements of ecological gradient analysis. Sculptured seeding prescribes planting two or more different mixtures of species within a single field, with each mixture tailored to a particular environmental site. A typical configuration might include one seed mix for hilltops, a second mix for poorly drained soils at the footslope, and a third mix for the intermediate midslopes. Selecting the proper species and number of species for such mixes requires knowledge of the plants, the local environment, and balancing economic and environmental considerations.

“Over-yielding” occurs when the mixture of two or more species yields more than expected based on monoculture yields of the same species. Ecological theory suggests that greater plant diversity should lead to higher yields because a variety of species can more fully utilize available resources than a single or small number of species (Tilman, 1999; Mulder et al., 2001). Some research on planted mixtures and monocultures (e.g. Tilman et al., 2001, 2006) has supported the theory that yield increased with diversity. However, other research has found yield of specific monocultures or simple mixtures often equals or exceeds high-diversity mixes (Jarchow and Liebman, 2012; Johnson et al., 2010; Picasso et al., 2008; Springer et al., 2001). In particular, mixtures that include a warm-season perennial grass (often switchgrass) and a high-yielding legume (such as alfalfa [*Medicago sativa*]) often yield more than monocultures of the same species or higher-diversity mixtures (DeHaan et al., 2010; Wang et al., 2010).

Thus, we designed an experiment in eastern South Dakota based on the ecological concept of gradient analysis and its application to revegetation through sculptured seeding, employing a monoculture of switchgrass as the constant treatment for comparison to all other treatments across the environmental gradient. Our primary objective was to: (1) determine if biomass yield was influenced, relative to a monoculture of switchgrass, by varying the ratio of the number of plants of switchgrass to the number of plants of three alternative native species with a constant plant density at each of three different landscape positions along an environmental gradient, and, at the same time and (2) compare monocultures of switchgrass to monocultures of the three alternative species for biomass yield at each of the three landscape positions.

## 2. Materials and methods

### 2.1. Site and experimental design

The experimental site was located on a privately-owned farm leased by EcoSun Prairie Farms, Inc. (Zilverberg et al., 2014) near Colman, SD (44.029, −96.850), within the Prairie Pothole region of the North American Tallgrass Prairie. The farm's topography is typical of the region: a rolling plain with numerous wetlands. Most of the farm had been planted to annual crops for the past century (Olson et al., 2014) and the plots used in our experiments were planted annually to corn from 2008 through 2011. Soils included Wentworth-Egan silty clay loams with 2–6% slopes, Dempster–Talmo complex with 2–9% slopes, and Worthing silty clay loam (Soil Survey Staff, 2013). Climate means from 1981 to 2010 were: annual precipitation, 686 mm; minimum temperature, 0 °C; and maximum temperature, 12 °C (NOAA, 2013) (Figs. 1 and 2).

Three blocks were established with different soil types (Table 1) and aspects (south-facing, west-facing, and east-facing). Each block contained three slope positions: shoulderslope, midslope, and footslope that corresponded to a different historic plant community. Measurements of historic vegetation at our site are not available, but vegetation most likely corresponded to Weaver's (1954) upland little bluestem community (shoulderslope), the transition from upland little bluestem community to lowland big bluestem community (midslope), and the transition from lowland big bluestem community to lowland sloughgrass (prairie cordgrass) community (footslope). Our research plots were ∼50 km north of Weaver's research prairies near Sioux Falls, SD, which were among the northernmost sites of his Tallgrass Prairie research (Weaver, 1968). On each slope, the midslope position was located exactly half way between the shoulderslope and footslope. From shoulderslope to footslope, mean horizontal distance and elevation change were 52 m and 2.33 m. All slope positions contained switchgrass and three alternative species: a warm-season grass, a cool-season grass, and a forb (Table 2).

Within each slope position were 10 plots, including a monoculture of switchgrass (1 plot), monocultures of each alternative species (3 plots), and each alternative species planted in a binary mixture with switchgrass such that switchgrass comprised 33% (3 plots) or 67% (3 plots) of the plants within the plot (Table 3). This design is a replacement series (Silvertown and Lovett Doust, 1993) wherein the ratio of the two species to each other varied but the total plant density was constant. This design assumed that competition among

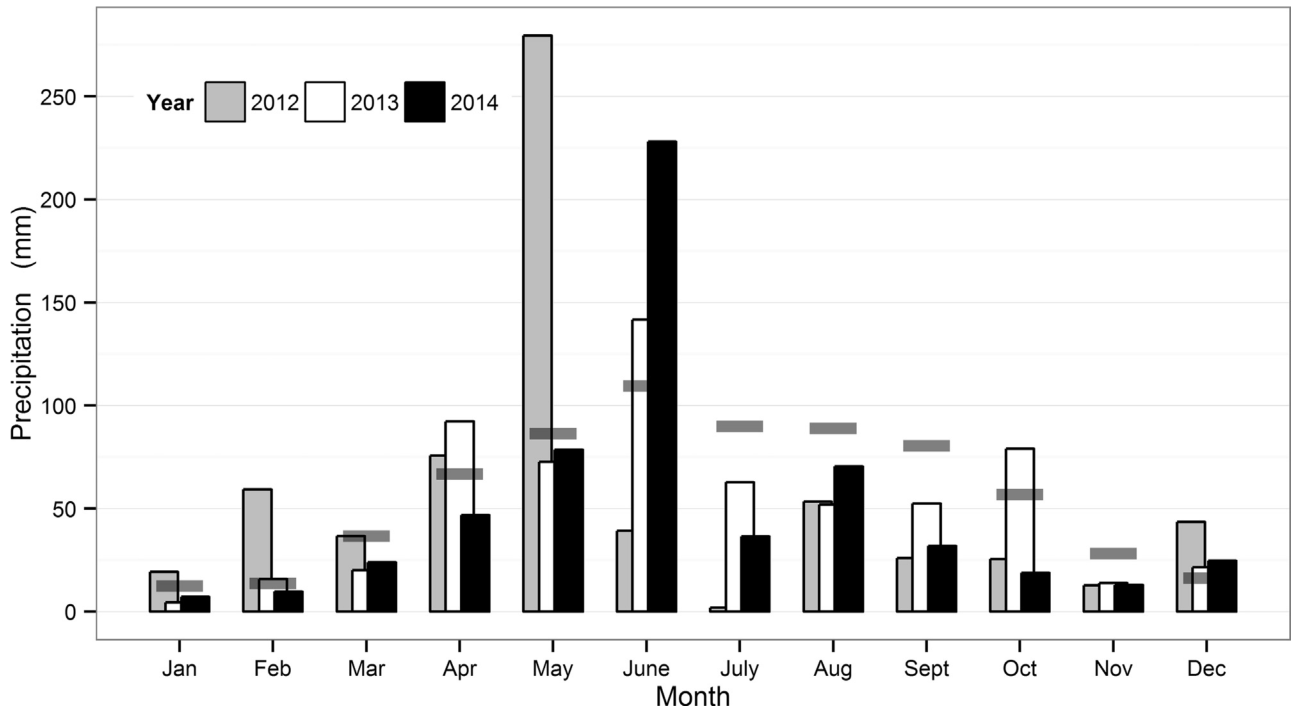


**Fig. 1.** Monthly precipitation totals at Flandreau, SD for three years. Long-term means (1981–2010; http://climate.sdstate.edu/archives/data/precipnormals.asp) are indicated by horizontal bars. Missing data for 14 days of September 2014 were filled in with data from Madison, SD, which is ~50 km from Flandreau. The experimental site was centrally located between Madison and Flandreau.

plants at a given mixture proportion would be the same regardless of plant density. Consequently, we chose a plant density based on Weaver's (1954) descriptions of basal cover in mature northern Tallgrass Prairie.

Plots measured 2.2 $m^2$ (1.6 × 1.4 m), were separated by a 0.9-m border, and plants were spaced approximately 0.3 m apart. At each slope position, the 10 treatments were randomly assigned to plots. Within each plot that contained two species, the location of individual plants was randomly assigned subject to the constraint that at least one of each species be present in each row and each column.

### 2.2. Selection of species

All entries were local or regional ecotypes that had undergone some selection for improved agronomic performance, except for cup plant (*Silphium perfoliatum*) and purple prairie clover (*Dalea purpurea*), which were not selected populations (Table 2). 'Sunburst,' the cultivar of switchgrass used, was selected from a natural population from southeastern South Dakota (Boe and Ross, 1998) but was shown to excel in yield and survival in both North and South Dakota (Berdahl et al., 2005; Tober et al., 2007).

For each landscape position, we selected one alternative species from each of three functional groups: $C_3$ grasses, $C_4$ grasses, and forbs. Different functional groups might avoid competition with one another, or even mutually facilitate growth in a number of ways, including variation in season of growth, root structure and depth, and the ability to symbiotically fix N. Species were selected based on their likely historic association with the landscape position and their potential for biomass yield at that position. In addition, all have utility as livestock forage, which is an important consideration because of the uncertainty regarding the future of ligno-cellulosic biofuels. Although we used selections or cultivars of the species rather than seed harvested from native remnants, all entries originated from Northern Great Plains populations, so all were adapted to the region (e.g., Boe and Ross, 1998). A brief description and justification of each follows.

Prairie cordgrass (footslope), big bluestem (midslope), and little bluestem (shoulderslope) were the $C_4$ grasses selected. All were common members of the tallgrass prairie, dominated their topographic positions (Weaver, 1954), and are recognized as valuable livestock forage when immature. Little bluestem was likely the most common historic upland plant species in the region where the experiment was conducted (Weaver, 1954). Prairie cordgrass and big bluestem have been previously evaluated as potential biofuel feedstock because of their high yields.

Western wheatgrass (*Pascopyrum smithii*; footslope), slender wheatgrass (*Elymus trachycaulus*; midslope), and green needlegrass (*Nassella viridula*; shoulderslope) were the $C_3$ grasses selected. Western wheatgrass is rhizomatous; slender wheatgrass and green needlegrass are both bunch grasses. All three are well-recognized as desirable forage species.

Cup plant (footslope), Canada milkvetch (*Astragalus canadensis*; midslope), and purple prairie clover (shoulderslope) were the forbs selected. Purple prairie clover and Canada milkvetch both have high aesthetic value due to their showy flowers, in addition to the contribution these legumes make through symbiotic nitrogen fixation. Both were recognized by Weaver (1954) as species that decrease in abundance when grazed because of high palatability. Canada milkvetch often behaves as a biennial and individuals may die or become very weak in the year after producing a heavy seed crop (Boe and Fluharty, 1998), but its ability to reseed allows the species to persist in mixed stands. Cup plant is a forb that tolerates mild flooding. Cup plant is highly digestible (Han et al., 2000), has long been used as forage in Asia, and has potential for use as a forage crop for cattle (Lehmkuhler et al., 1997), although its use for that purpose is uncommon in the U.S. The two legumes and cup plant are valued for providing habitat and food for pollinators.

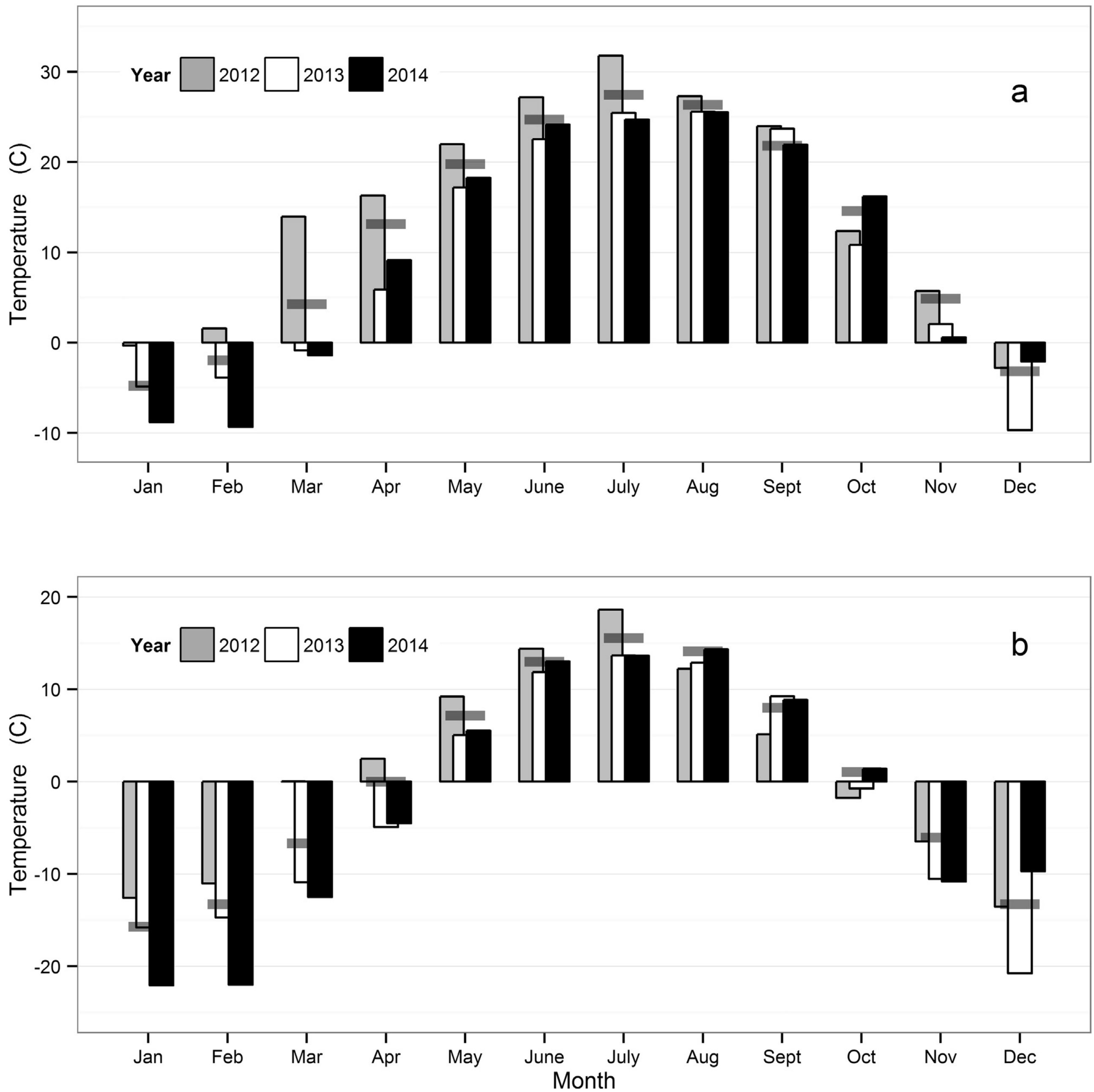


**Fig. 2.** Mean monthly high (a) and low (b) temperatures for the three years of the experiment at Flandreau, SD. Long-term means (1981–2010; http://climate.sdstate.edu/archives/data/tempnormals.asp) are indicated by horizontal bars. Missing data for 14 days of September 2014 were filled in with data from Madison, SD. Growing degree days through 30 September with a base temperature of 0 for the three years were 3441 (2012), 2644 (2013), and 2778 (2014).

### 2.3. Management and sampling

All plants were started from seed, grown in cone-shaped containers ("cone-tainers"; Stuewe & Sons, Tangent, OR; conetainer size was 2.5 cm diameter × 16 cm depth) in the greenhouse, and acclimated before being transplanted into field plots between 11 and 18 June, 2012. All plants were initially spot-irrigated at transplanting to aid establishment. Due to drought conditions during the establishment year, plants were also spot-irrigated as needed during July when symptoms of moisture stress were

**Table 1**
Slope (change in elevation from top to bottom of plot) and soil properties of the three landscape positions at the 0–15 cm depth, averaged across three experimental blocks.

| Landscape position | Slope (mm$^{-1}$) | $NO_3$-N (ppm) | Olsen P (ppm) | *K* (ppm) | pH | Salts 1:1 (mmho cm$^{-1}$) | Texture Sand (%) | Silt (%) | Clay (%) |
|---|---|---|---|---|---|---|---|---|---|
| Shoulderslope | 0.031 | 2.9 | 3 | 126 | 7.0 | 0.33 | 41 | 28 | 31 |
| Midslope | 0.054 | 4.3 | 5 | 126 | 6.7 | 0.20 | 39 | 30 | 30 |
| Footslope | 0.022 | 2.5 | 8 | 147 | 7.1 | 0.33 | 30 | 38 | 31 |

**Table 2**
Species planted at each slope position. 'Sunburst' switchgrass was used at all slope positions.

| Slope position | Warm-season grass | Cool-season grass | Forb |
|---|---|---|---|
| Shoulder | 'Badlands ecotype'<br>little bluestem (*Schizachyrium scoparium*) | 'Lodorm'<br>green needlegrass (*Nassella viridula*) | Purple prairie clover (*Dalea purpurea*)[a] |
| Mid | 'Sunnyview'<br>big bluestem (*Andropogon gerardii*) | 'Primar'<br>slender wheatgrass (*Elymus trachycaulus*) | 'Sunrise'<br>Canada milkvetch (*Astragalus canadensis*) |
| Foot | 'Prairie Farm'<br>prairie cordgrass (*Spartina pectinata*) | 'Rodan'<br>western wheatgrass (*Pascopyrum smithii*) | Cup plant (*Silphium perfoliatum*)[b] |

[a] Natural germplasm from the Bismarck Plant Materials Center.
[b] Population developed at South Dakota State University.

evident. Plants were not irrigated in subsequent years. About 2% of the plants died during the transplant year and were replaced with greenhouse-grown plants during the transplant year. Plants that died after the establishment year were not replaced. Weeds were removed by hoeing or pulling.

Individual plots were harvested by hand with a rice knife at a 12-cm stubble height and separated by species on 30 September–2 October, 2013 and 2–4 October, 2014. Fresh biomass of each species in a plot was weighed individually in the field. Grab subsamples of each species were collected, dried to constant weight, and used to calculate percentage dry matter. All weights are reported on a dry matter basis. After harvest in each of the production years, percentage basal ground cover was estimated for two randomly selected plants of each species in each plot. For each plant, cover was visually estimated within a quadrat measuring $41 \times 46$ cm, the area allocated for each plant. Where the rhizomatous western wheatgrass or prairie cordgrass had invaded the space allocated to a switchgrass plant, the ground cover for switchgrass and the invading species were estimated separately. Tillers of rhizomatous grasses within 4 cm of one another were considered to form a cluster that included the space between them.

### 2.4. Statistical analysis

For biomass production, each landscape position was analyzed independently as a randomized complete block design with treatment as a fixed factor in the main plot and block as random. Year was included as a repeated measure, and the interaction of year and treatment was also included. Mean separation was applied using Fisher's LSD at the highest level of interaction or treatment found to be significant. In addition, a separate analysis was conducted on the switchgrass monoculture treatments. This analysis included landscape position as the main plot treatment, block as a random effect, and year as a repeated measure. The year by treatment interaction was included, and mean separation proceeded as described in the first analysis. Basal area of each species was independently analyzed with a randomized block design, using a random effect of block and a fixed effect of year. All basal area subsamples of a companion species in a given block were averaged before analysis. For switchgrass, a separate analysis was conducted for each of the three landscape positions. All estimates of switchgrass basal area at a given landscape position were averaged before analysis, regardless of the identity of the companion species.

To test for over- and under-yielding, we ran one regression for each companion species. Each regression included total plot biomass (dependent variable), block (random effect), portion of companion species (fixed effect), and portion of companion species squared (fixed effect). For each species in these regressions, we used the companion monoculture, the two mixtures (33 or 67%) containing the companion, and the switchgrass monoculture from the same landscape position. When the quadratic term was positive (negative) and significant, we interpreted it as over-yielding (under-yielding).

All statistical tests were carried out at $p \leq 0.05$ using the lmer (Bates et al., 2013) and nlme (Pinheiro et al., 2013) packages of R version 3.0.2 (R Core Team, 2013). All figures were produced with ggplot2 version 0.9.3 (Wickham, 2009).

## 3. Results and discussion

### 3.1. Weather and establishment

Late winter and early spring of 2012, the establishment year, were abnormally wet and temperatures were abnormally high (Figs. 1 and 2). The heat continued through midsummer; precipitation was also well below normal during summer. By the end of the first growing season, all plots were considered well established except for one replication of the green needlegrass monoculture, which had several plants that displayed poor vigor. In contrast to the establishment year, the two production years, 2013 and 2014, were below the mean for temperature most of the year. They were also drier than average for most months, with the notable exception of a wet June in both years (29 and 108% greater than normal, respectively).

### 3.2. Basal area

Basal area of little bluestem increased from 15% in 2013 to 21% in 2014 ($p < 0.05$; Table 4). Similarly, cup plant increased from 9 to 15%, and prairie cordgrass from 28 to 65% ($p < 0.05$). Switchgrass basal area increased from 14 to 26% at the shoulderslope and 10–21% at the midslope ($p < 0.05$). Mean basal area of all other species except Canada milkvetch increased numerically but not statistically ($p > 0.05$) from 2013 to 2014. Thus, after two years, basal area at the shoulderslope and midslope positions in this experiment resembled basal area of historic Tallgrass Prairie, as measured by Weaver (1954) (Table 4).

**Table 3**
Example of plot layout. The following plots were at the midslope of block 3.

| | | | | |
|---|---|---|---|---|
| 100% big bluestem | 67% Canada milkvetch<br>33% switchgrass | 100% Canada milkvetch | 100% switchgrass | 33% big bluestem<br>67% switchgrass |
| 100% slender wheatgrass | 67% big bluestem<br>33% switchgrass | 67% slender wheatgrass<br>33% switchgrass | 33% slender wheatgrass<br>67% switchgrass | 33% Canada milkvetch<br>67% switchgrass |

**Table 4**
Basal area of companion species that were combined with switchgrass in binary mixtures at three landscape positions. For rhizomatous species, the first value is basal area within the original plant's area, and the second value is the basal area of the same species inside switchgrass areas. The *p*-values are for comparison of the two years for a given species.

| Species | Year | | *p*-value | Mean basal cover of historic tallgrass prairie communities |
|---|---|---|---|---|
| | 2013 | 2014 | | |
| Shoulderslope | | | | |
| Purple prairie clover | 5 | 7 | 0.09 | Green needlegrass community 10.9% |
| Green needlegrass | 11 | 14 | 0.13 | |
| Little bluestem | 15 | 21 | 0.02 | Little bluestem community 15.3% |
| Switchgrass | 14 | 26 | 0.01 | [43] |
| Midslope | | | | |
| Canada milkvetch | 7 | – | – | Big bluestem community 13.3% |
| Slender wheatgrass | 9 | 19 | 0.16 | [43] |
| Big bluestem | 10 | 16 | 0.13 | |
| Switchgrass | 10 | 21 | 0.04 | |
| Footslope | | | | |
| Cup plant | 9 | 15 | 0.03 | Prairie cordgrass (species only, not the entire community) 1–3% |
| Western wheatgrass | 43 (8) | 69 (23) | 0.16 (0.03) | [14] |
| Prairie cordgrass | 28 (0) | 65 (14) | 0.02 (0.02) | |
| Switchgrass | 10 | 16 | 0.09 | |

Most estimates of success of establishment for seeded stands of native grasses utilize plants per unit area (Cornelius, 1944) or frequency of grid cells occupied by at least 1 tiller (Vogel and Masters, 2001). Cornelius (1944) determined that 10 plants $m^{-2}$ the year after planting was a good stand for native warm-season grasses, whereas Schmer et al. (2006) determined that a good stand of seeded switchgrass would have at least 1 tiller in 40% of the cells in a grid composed of twenty-five 0.02-$m^2$ cells in the second year after planting. In contrast, since our study utilized equidistant-spaced transplants to facilitate initial equal resource availability for individual plants, Weaver's data are presented to suggest the space allowed for each plant was reasonable, but this does not imply a direct relationship with population density in natural Tallgrass Prairie communities.

We are not aware of comparable measurements of historic prairie communities for the footslope. However, Weaver (1954) stated that, within the prairie cordgrass community, prairie cordgrass stems only covered 1–3% of the soil surface. Weaver (1954) does not state the basal area of the entire prairie cordgrass community. Regardless, our values for prairie cordgrass were much greater than those of Weaver (1954), presumably due to difference in methodology. Weaver measured the area of each individual stem and totaled the area of all stems. In contrast, for strongly rhizomatous species (i.e., prairie cordgrass and western wheatgrass), we considered clusters of tillers with interstitial spaces <4 cm between nearest-neighbor tillers to cover the entire contiguous area.

### 3.3. Annual biomass production by landscape position

At the shoulderslope position, the main effects of year and treatment were both significant but the year × treatment interaction was not (Table 5). Mean biomass yield increased from 8.4 Mg $ha^{-1}$ in 2013 to 10.0 Mg $ha^{-1}$ in 2014. At the shoulderslope, the highest-yielding treatments were those containing little bluestem, those containing at least 67% switchgrass, and the 67% purple prairie clover mixture (Fig. 3). The lowest-yielding treatments were monocultures of green needlegrass (4.1 Mg $ha^{-1}$), purple prairie clover (4.4 Mg $ha^{-1}$), and the 67% green needlegrass mixture (7.6 Mg $ha^{-1}$).

The year × treatment mean square was significant for biomass for both midslope and footslope landscape positions (Table 5). In general, in both 2013 and 2014, the highest-yielding treatments at the midslope were those containing big bluestem (Figs. 4 and 5). In 2013, biomass yield of the switchgrass monoculture was only exceeded by that of the big bluestem monoculture. All of the treatments containing Canada milkvetch or slender wheatgrass produced less biomass than the switchgrass monoculture and those containing big bluestem. However, whereas monoculture grass yields at the midslope did not change across years ($p > 0.05$), Canada milkvetch monoculture yield declined ($p < 0.05$) from the second (2013) to third (2014) year after planting.

At the footslope position in 2013, treatments containing at least 67% prairie cordgrass or cup plant produced more biomass than the switchgrass monoculture or the treatments containing western wheatgrass. Biomass yields of the cup plant and prairie cordgrass monocultures and the 67% cup plant mixture were similar. The cup plant monoculture out-yielded all treatments except the 67% cup plant mixture and the prairie cordgrass monoculture (Fig. 4). Biomass yield of treatments containing cup plant declined dramatically in 2014 due to herbicide damage (Fig. 5; $p < 0.05$), consequently producing less biomass than treatments containing prairie cordgrass. In 2014, treatments containing prairie cordgrass

**Table 5**
Mean squares from analysis of variance for biomass for two production years, 2013 and 2014. Separate analyses were conducted at each landscape position. Treatments were switchgrass monocultures, monocultures of alternative species, and binary mixtures of switchgrass with alternative species. In addition, switchgrass monocultures were compared to one another across landscape positions.

| Source of variation | Degrees of freedom | Mean squares by landscape position | | |
|---|---|---|---|---|
| | | Shoulderslope | Midslope | Footslope |
| Treatment (T) | 9 | 54.5[a] | 143[a] | 74[a] |
| $Error_T$ | 18 | 10.7 | 2.5 | 15 |
| Year (Y) | 1 | 36.8[a] | 11.6[a] | 656[a] |
| T × Y | 9 | 2.0 | 8.5[a] | 66[a] |
| $Error_Y$ | 20 | 1.9 | 1.5 | 15 |

| Source of variation | Degrees of freedom | Switchgrass monoculture |
|---|---|---|
| Landscape position (P) | 2 | 3.7 |
| $Error_P$ | 4 | 2.6 |
| Year (Y) | 1 | 5.3 |
| P × Y | 2 | 3.5 |
| $Error_Y$ | 6 | 1.8 |

[a] Significant at the 0.01 level.

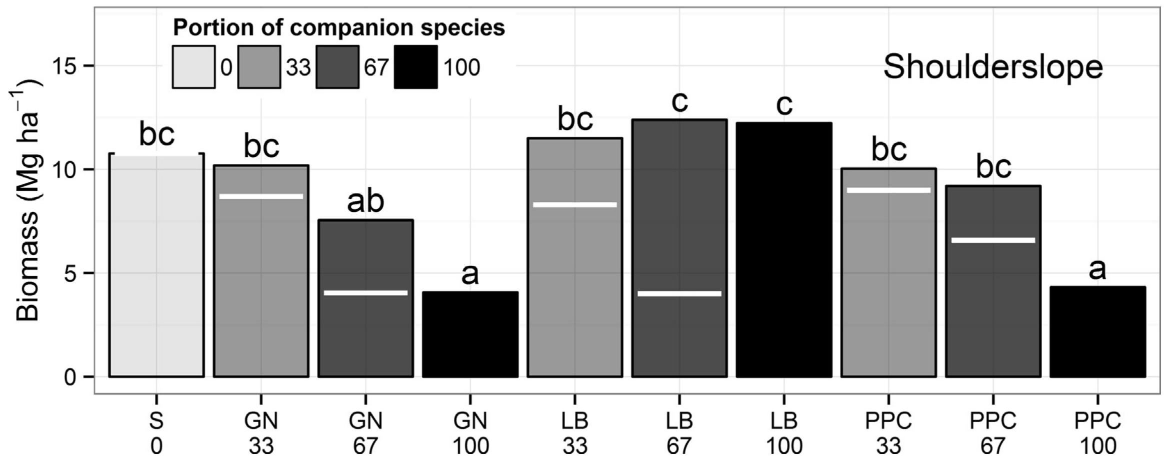


**Fig. 3.** Biomass of monocultures and binary mixtures of native plant species at the shoulderslope position, averaged across 2013–2014, the first two production years. Mixtures included switchgrass and a companion species that composed 33 or 67% of the total plants. All plots included the same number of plants. Labels on the *x*-axis indicate the species (S, Switchgrass; GN, Green Needlegrass; LB, Little Bluestem; and PPC, Purple Prairie Clover) and the percentage of the plants that were from the companion species. For mixture plots, the area below the horizontal white line represents switchgrass biomass, and the area above the line represents companion biomass. Two bars with the same letter above them are not statistically different ($p > 0.05$).

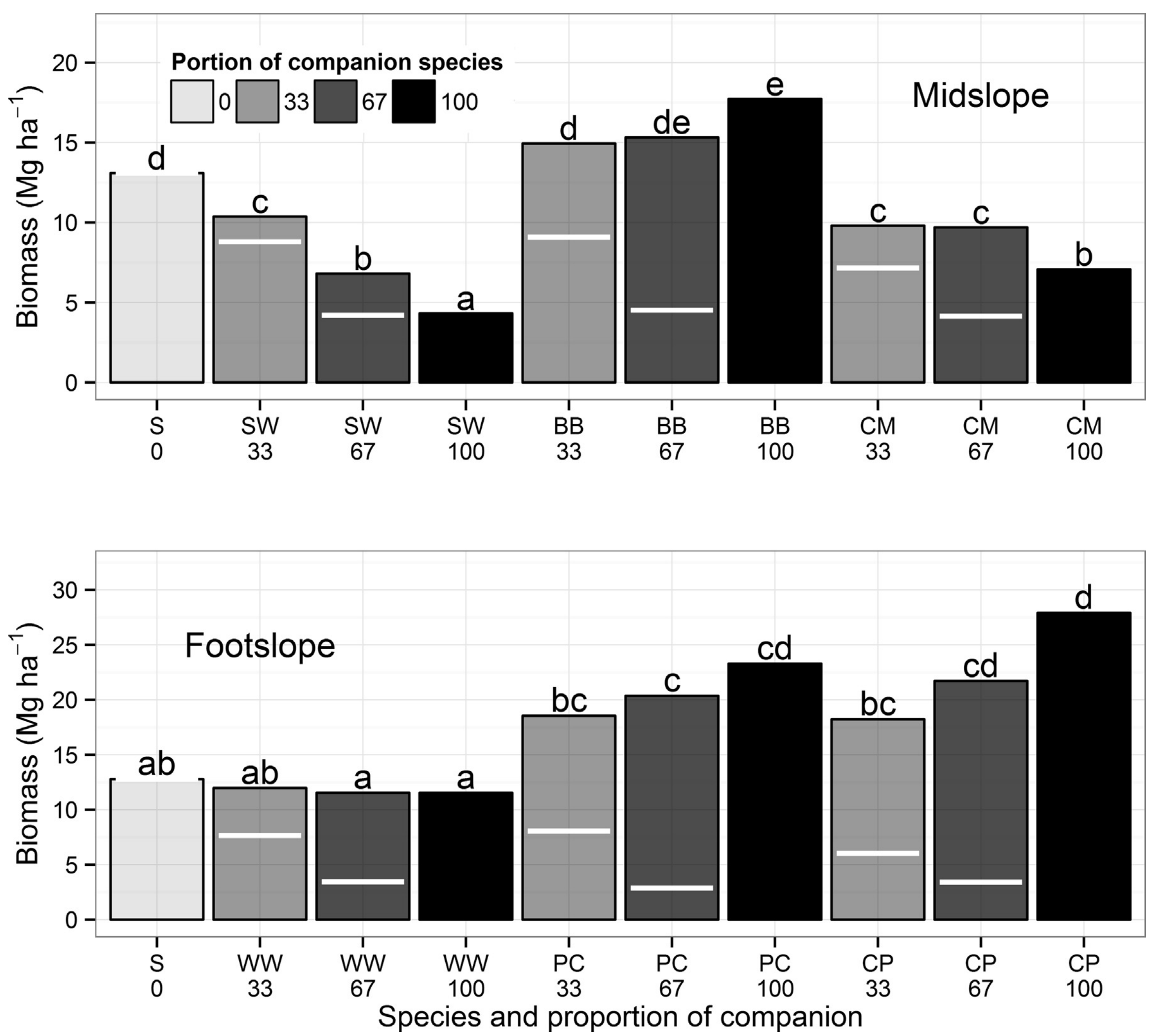


**Fig. 4.** Biomass of monocultures and binary mixtures of native plant species at two landscape positions (midslope and footslope) in 2013, the first production year. Mixtures included switchgrass and a companion species that composed 33 or 67% of the total plants. All plots included the same number of plants. Labels on the *x*-axis indicate the species (S, Switchgrass; SW, Slender Wheatgrass; BB, Big Bluestem; CM, Canada Milkvetch; WW, Western Wheatgrass; PC, Prairie Cordgrass; and CP, Cup Plant) and the percentage of the plants that were from the companion species. For mixture plots, the area below the horizontal white line represents switchgrass biomass, and the area above the line represents companion biomass. Two bars from the same landscape position with the same letter above them are not statistically different ($p > 0.05$).

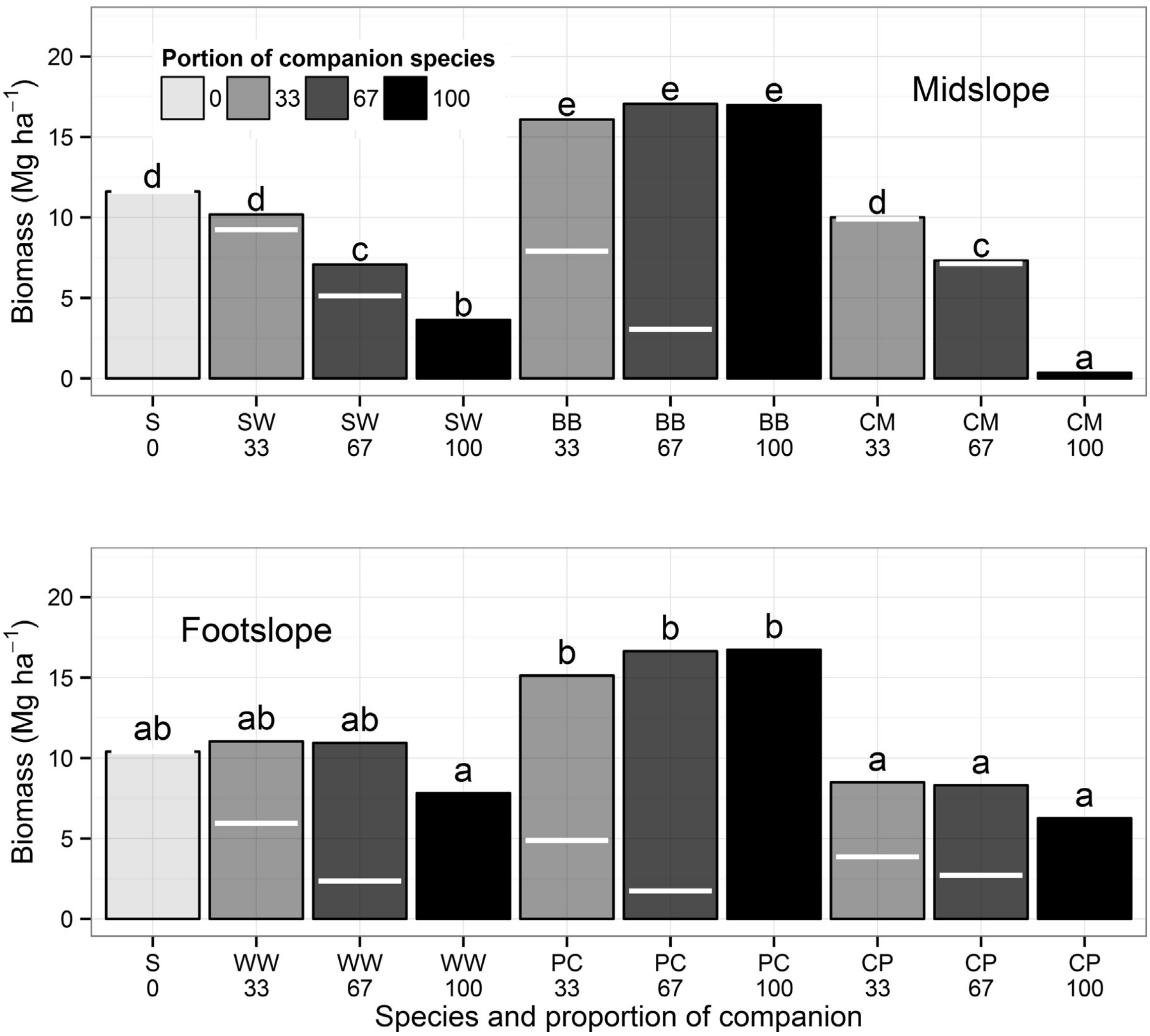


**Fig. 5.** Biomass of monocultures and binary mixtures of native plant species at two landscape positions (midslope and footslope) in 2014, the second production year. Mixtures included switchgrass and a companion species that composed 33 or 67% of the total plants. All plots included the same number of plants. Labels on the *x*-axis indicate the species (S, Switchgrass; SW, Slender Wheatgrass; BB, Big Bluestem; CM, Canada Milkvetch; WW, Western Wheatgrass; PC, Prairie Cordgrass; and CP, Cup Plant) and the percentage of the plants that were from the companion species. For mixture plots, the area below the horizontal white line represents switchgrass biomass, and the area above the line represents companion biomass. Two bars from the same landscape position with the same letter above them are not statistically different ($p > 0.05$). Cup plant yield was reduced by herbicide damage in 2014.

produced similar amounts of biomass to the switchgrass monoculture and the western wheatgrass mixtures, but more biomass than the western wheatgrass monoculture (Fig. 5).

At all landscape positions and in both production years, multiple treatments yielded at least as much biomass as switchgrass monocultures. This demonstrated that matching specific plant species to locations where they are adapted in a heterogeneous field landscape can result in comparable to greater biomass yields and always greater small-scale and landscape-scale diversity than a switchgrass monoculture. Matching monocultures and/or seed mixes to landscape position, also referred to as sculptured seeding (Jacobson et al., 1994), is not widely used for revegetation, forage, and biofuel plantings in the eastern Dakotas. However, a heterogeneous landscape intended to be managed for sustainable long-term dedicated biomass for biofuel is ideally suited for sculptured seeding because: (1) planting several monocultures or several simple mixtures, which is more complicated than planting a monoculture, ideally need only occur once per 10 or more years, although the species composition of even simple mixtures is likely to change over this time period and (2) depending upon the technology used to convert biomass to biofuel, the entire landscape could be harvested in bulk rather than separately by species.

With one exception, the highest biomass yields came from alternative $C_4$ grasses or mixtures dominated by switchgrass, also a $C_4$ grass. This was not surprising, since the $C_4$ grasses used in this experiment historically dominated the northern Tallgrass Prairie (Weaver, 1954) and their potential productivity is well known (e.g., Boe et al., 2004, 2013). In addition, the timing of biomass sampling, which occurred in early October, favored $C_4$ grasses over $C_3$ because, although the $C_3$ grasses had some new tiller growth beginning in September, their initial spring growth had senesced and deteriorated after seed maturity in July. All $C_3$ grasses greened-up at least 30 days earlier than switchgrass (Supplementary Fig. 1). Among the $C_3$ grass monocultures, only western wheatgrass performed comparably to switchgrass. When combined with 67% switchgrass, however, all three $C_3$ species produced yields not different from a switchgrass monoculture in at least one of the two production years. About 600 km northwest of our study site, introduced cool-season grass monocultures or their mixtures with switchgrass outyielded switchgrass monocultures (Wang et al., 2014).

The exception to the dominance by $C_4$ grasses was the performance in 2013 of cup plant, a large forb in the sunflower family (i.e., Asteraceae), at the footslope. The cup plant-dominated plots outyielded all treatments except the prairie cordgrass-dominated treatments, from which they did not differ (Fig. 4). Unfortunately, in 2014, cup plant was morphologically contorted by a herbicide applied to a neighboring field. Despite this damage, cup plant yields did not differ from switchgrass in 2014 (Fig. 5). Monocultures of the other two forbs yielded less than monocultures of switchgrass. Canada milkvetch performed reasonably well in 2013, but because of its biennial-like growth habit, it had all but disappeared in 2014. In mixture, the 2014 decline of Canada milkvetch corresponded with an increase in switchgrass biomass. Unlike Canada milkvetch, purple prairie clover maintained its productivity throughout the experiment, but, similar to Canada milkvetch in 2014, purple prairie clover made up only a small portion of the treatment biomass when mixed with switchgrass.

One of the reasons why switchgrass is among the best options for producing biomass is because it yields well across a range of environmental conditions when planted in monoculture. In this study, switchgrass monoculture biomass did not differ across landscape positions ($p = 0.34$; shoulder, 10.8 Mg ha$^{-1}$ [sd = 1.3]; midslope, 12.4 [sd = 1.3]; footslope, 11.6 [sd = 2.1]). Yields were intermediate compared to yields of drill-seeded switchgrass in two experiments at the same site (Zilverberg et al., 2014). However, our results also demonstrated that switchgrass monocultures did not necessarily maximize biomass production at each of the three landscape positions, compared to alternatives. For example, at the shoulderslope, little bluestem, purple prairie clover, and green needlegrass could be mixed with switchgrass without losing yield (Fig. 3). At the midslope, yield was maximized by big bluestem mixtures or monocultures (Figs. 4 and 5). At the footslope, yield was maximized by mixtures or monocultures of cup plant or prairie cordgrass (Figs. 4 and 5).

### 3.4. Over- and under-yielding

In 2013, the $p$-value of the quadratic term in the regressions was non-significant for all companion species, indicating that no over-yielding or under-yielding occurred (Fig. 6). The lack of any significant effects might be explained by the immaturity of the stands—that is to say, individual plants had not yet expanded enough to exert significant positive or negative effects on their neighbors. However, in 2014, mixtures of switchgrass with western wheatgrass, slender wheatgrass, Canada milkvetch, and big bluestem demonstrated over-yielding (Fig. 6). Switchgrass had increased its basal area by 2014 (Table 4), making plots more “crowded”, and presumably resulting in more inter-specific interaction. Canada milkvetch was a special case in 2014, since the few surviving plants displayed low vigor, turning this species' mixtures into virtual switchgrass monocultures. Thus, the Canada milkvetch mixtures may have over-yielded because individual switchgrass plants experienced little competition for resources.

For western wheatgrass and slender wheatgrass, over-yielding might be explained by partitioning growth periods into different parts of the growing season. These two cool-season grasses greened-up ~40 days earlier than switchgrass (Supplementary Fig. S1). Green needlegrass, the only cool-season grass that did not over-yield, was the last cool-season grass to green-up. Over-yielding by big bluestem is not easily explained, since it belongs to the same functional group as switchgrass. Big bluestem and switchgrass greened-up at approximately the same date and switchgrass advanced in maturity only slightly more rapidly (Supplementary Fig. S1). Cardinale et al. (2007) found that it takes ~5 years for higher-diversity plantings to outcompete monocultures. It is not clear if over-yielding would also increase with our simple mixtures, given more time.

### 3.5. Considerations for application at the field scale

At a constant plant density, switchgrass monocultures failed to outproduce several other native plant monocultures and binary mixtures that included switchgrass. One might then reasonably ask, where and when would a monoculture of switchgrass be preferred for plantings that are intended for environmental benefits as well as biofuel production? There are some good reasons, most of which are agronomic. First, because of the free-flowing nature of its seed, planting would be simpler, more precise, and seed cost would most likely be less. Second, although switchgrass monocultures have required tailored management to sustain long-term yields and stand persistence (e.g., Mulkey et al., 2006; Lee et al., 2007), weed control is easier for fields containing a single functional group because it allows a broader range of choice in timing and type of herbicide application. Third, uniform feedstock is desirable, especially if destined for ethanol production. However, biomass feedstock can be converted to fuel via other means, such as combustion or other thermal processes to produce bio-oils, syngas, and biochar (Bridgwater, 2012), where feedstock heterogeneity would be less problematic (Nackley, 2015).

The environmental benefits of diversity are well documented, impacting everything from soils, water relations, microscopic life within soils, and aboveground wildlife (e.g., Myers, 1996; Brussaard et al., 2007). Thus, it is reasonable to assume that using sculptured seeding to increase diversity would benefit the environment, but there are as yet few data comparing environmental impacts of alternative perennial vegetation managed for biofuel production in the Tallgrass Prairie (Burkhalter, 2013). Based on our results, given the biomass yield benefits expected to be provided by sculptured seeding, this approach may appeal to landowners who want to produce a profitable biofuel commodity and who are concerned with natural resource conservation. Regarding the application of our experimental results to an entire field, consider the following: if one selected for a field the highest-yielding treatment at each landscape position, averaged over the two years, the advantage over a switchgrass monoculture would be: shoulderslope, +1.6 Mg ha$^{-1}$ using 67% little bluestem; midslope, +5.0 Mg ha$^{-1}$ using a big bluestem monoculture; and footslope, +8.4 Mg ha$^{-1}$ using a prairie cordgrass monoculture. This strategy would place four plant species in the field rather than one, but it would be lacking in functional diversity, since all four are $C_4$ grasses. If one had the goal of increasing functional diversity rather than maximizing yield, the forbs and $C_3$ grasses could be mixed with switchgrass and little or no yield would be lost relative to a switchgrass monoculture. Regardless of the approach used, static combinations of binary mixtures will lack the degree of diversity present in historic and remnant Tallgrass Prairie, even at the landscape scale.

Areas where further research is needed are evaluation and breeding of additional native prairie species to test their suitability and develop their potential for biomass production in monoculture and mixture, evaluation of additional binary combinations (e.g., prairie cordgrass with cup plant), evaluation of more complex mixtures under controlled conditions, similar experiments conducted in more regions, and evaluation of treatments at the field scale and longer time frames. As in this experiment, switchgrass would be an ideal standard against which to judge alternatives.

Characteristics determining the competitive ability of established plants include phenology, lateral spread, height, and response to stress and damage (Grime, 1979). For the mixtures

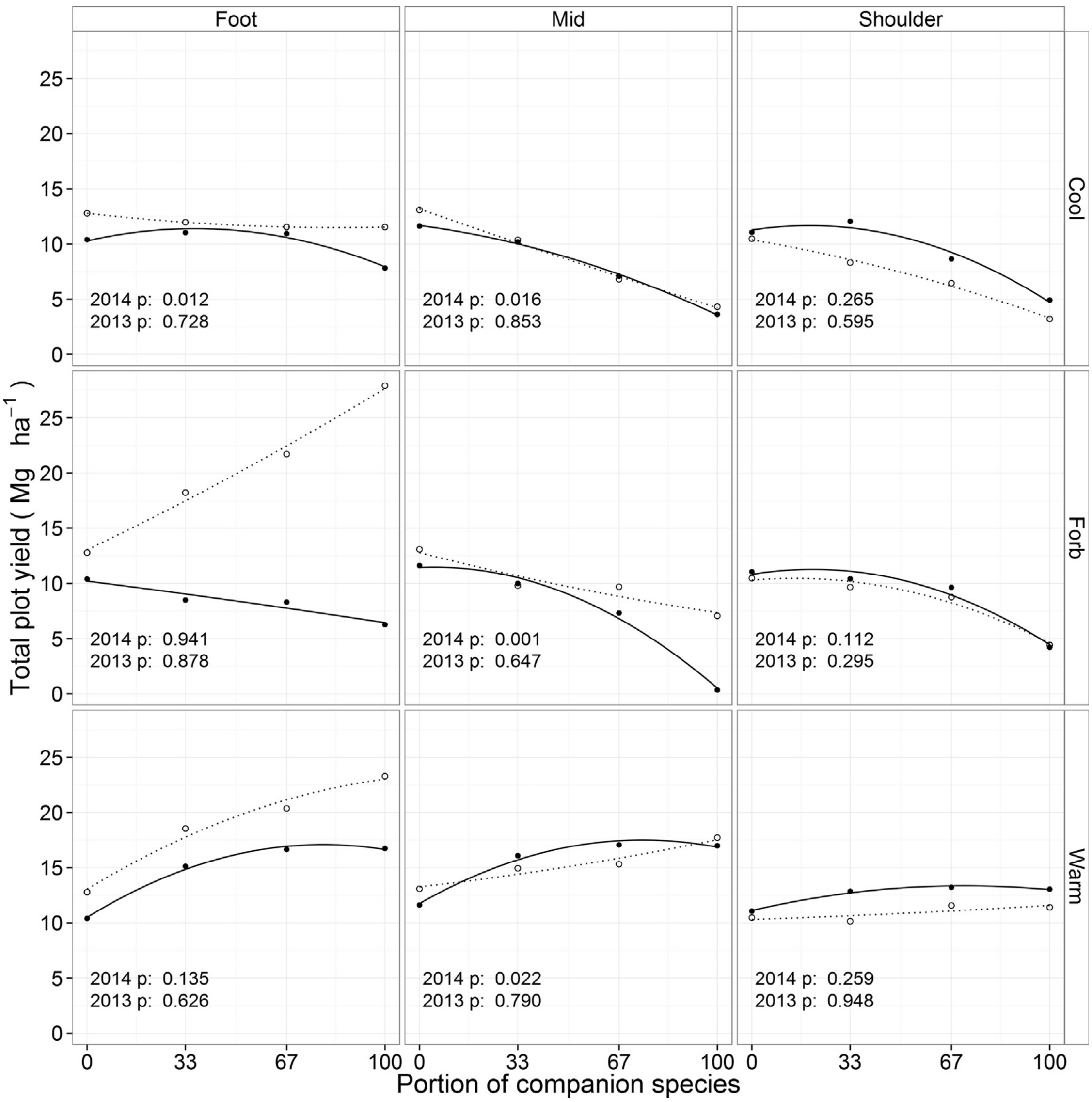


**Fig. 6.** Quadratic regression lines indicating over- or under-yielding of mixtures. Lines are drawn from switchgrass monocultures on left (0% companion), through the mixtures (33 or 67% companion), to the companion monocultures on right (100% companion). Solid lines indicate 2014. Dotted lines indicate 2013. *p*-values for the quadratic term are displayed on each graph. Significant *p*-values ($<0.05$) indicate overyielding. Species at each landscape position were (1) shoulderslope: green needlegrass (cool), purple prairie clover (forb), little bluestem (warm); (2) Midslope: slender wheatgrass (cool), Canada milkvetch (forb), big bluestem (warm); and (3) Footslope: western wheatgrass (cool), cup plant (forb), prairie cordgrass (warm).

of $C_4$ grasses, phenology was not a factor in the present experiment because populations of all four species had similar latitudinal origins. However, at all three landscape positions, the alternative $C_4$ grasses have superior lateral spread, compared to switchgrass (Branson, 1953; Weaver, 1954; Rechenthin, 1956). At the footslope and midslope, the alternative $C_4$ grasses have a height advantage over switchgrass, but switchgrass has the advantage over little bluestem at the shoulderslope (Supplementary Fig. S2). At the footslope, prairie cordgrass is more tolerant of flooding and salinity than switchgrass (Weaver, 1954). At the midslope and shoulderslope, the bluestems are also more tolerant than switchgrass to abiotic and biotic stresses and damage (Branson, 1953; Rechenthin, 1956).

Thus, if one seeded the $C_4$ grasses from our study (i.e., big bluestem, little bluestem, and prairie cordgrass) in binary mixtures at the field scale, it is likely that there would be a period of several years where, depending on the seeding rate of each species, switchgrass and the companion species would co-inhabit each of the three landscape positions. However, maintaining a permanent mixture composed of comparable numbers of individuals of each species would not be expected. Eventually, the bluestems would prevail at their adapted landscape positions, as would prairie cordgrass at the footslope (Boe, unpublished data, 2000–2008). Indeed, this has already begun happening in our experiment after just three years. From 2013 to 2014, the ratio of the biomass of the alternative species to switchgrass in a given mixture increased for all $C_4$ grasses: little bluestem went from 1.2 to 1.5, big bluestem from 1.6 to 3.3, and prairie cordgrass from 3.9 to 5.8. Dominance by a single species was a common feature of the Tallgrass prairies Weaver (1968) studied, where big bluestem and little bluestem usually constituted 75% of the basal area. For experimental reasons, our plots were managed to maintain the one or two species originally planted in each plot, but in field-scale plantings, non-planted desirable species could be allowed to colonize and remain in order to increase diversity. This approach was taken with regard to wetlands planted to prairie cordgrass on the experimental farm (Zilverberg et al., 2014).

The relatively consistent performance of the switchgrass monoculture across the environmental gradient in this study reinforced the widespread acceptance of the species' potential as a biomass feedstock for heterogeneous landscapes. However, because switchgrass imparted no biomass yield advantage over little bluestem and was out-yielded by big bluestem and prairie cordgrass, its usefulness in the northern Tallgrass Prairie region in low input cellulosic biomass production systems may be more as a short-term contributor to biodiversity rather than a major contributor to long-term biomass production in mixed plantings.

Unfortunately few studies have looked at biomass production from monoculture stands of switchgrass and other native grasses older than five years, so little is known about their comparative long-term productivity and sustainability in single-cut biomass production systems. However, our results indicated that, at least in the short term, growing numerous monoculture patches of various adapted species at their appropriate positions on heterogeneous landscapes could maximize biomass yield and increase field-scale diversity relative to switchgrass monocultures. Diversity could be further increased using numerous simple mixtures in place of monocultures, with little or no sacrifice of biomass yield.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2015.09.006.

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