Native Perennial Grassland Species for Bioenergy: Establishment and Biomass Productivity

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Biofuels

ABSTRACT

Proposed perennial bioenergy cropping systems include both native grass monocultures and polycultures of grasses and forbs. We determined the effect of species richness and composition on establishment and initial biomass production of native plant polycultures. Twelve treatments with varying levels of species richness (1–24 species) were established. Establishment success and yield varied over eight locations. The number of species established in polyculture increased linearly as the number of species seeded increased. Average biomass yield ranged from 1.2 to 6.0 Mg ha^{-1} with the highest yielding treatments being grass monocultures or an eight species grass–legume mixture. An increase in species richness from one to eight species increased yield an average of 28%, but increasing species richness from 8 to 12 or 24 species had no yield advantage at most locations. Early successional species, Canada milkvetch (*Astragalus canadensis* L.) and Maximilian sunflower (*Helianthus maximilian* Schrad.), were dominant in mixtures and contributed a majority of the biomass to the yield. Even in high diversity plots, biomass was from only a few plant species with a single species dominating the mixture. Our results suggest that selected low diversity mixtures (one to five species) likely offer the best combination of species establishment and high yield during stand establishment. However, we expect that early successional species that were dominant during the establishment phase of our experiment will contribute less biomass as stands mature and later successional species will become dominant and provide greater biomass.

Increasing worldwide demand for energy and the growing concern over global climate change have led to a surge in renewable energy research. This research encompasses a wide variety of initiatives aimed at finding reliable, sustainable, domestic fuel sources that reduce dependence on fossil fuels and minimize greenhouse gas emissions and other negative environmental impacts. The *Energy Independence and Security Act of 2007* (EISA) requires 136 billion L of renewable transportation fuels be available in the United States by 2022, and 60 billion L will be from advanced cellulosic sources. Achieving these goals will require the development of reliable large-scale production of diverse energy feed stocks.

The development of corn (*Zea mays* L.) grain ethanol brought renewable energy into the public arena and raised awareness about the potential for development of domestic energy sources (Solomon et al., 2007). However, growing concern over the environmental impact and economic viability of corn grain ethanol has prompted many to advocate for "second generation" bio-energy crops (Sanderson and Adler, 2008; Sarath et al., 2008). The most extensively studied of these crops are switchgrass (*Panicum virgatum L.*), reed canarygrass (*Phalaris arundinacea L.*), miscanthus x giganteus (*L.*), and alfalfa (*Medicago sativa L.*) (Sanderson and Adler, 2008). The cellulose, hemicellulose, and lignin components of these crops can be converted to energy-rich gases or liquid fuels and used to generate electricity or synthesize transportation fuels (Downing et al., 1995; McLaughlin et al., 2002; Parrish and Fike, 2005).

While many herbaceous and woody crops are being considered as next generation biofuels, perennial grasses have received the most research attention (McLaughlin and Walsh, 1998). Perennial grasses, specifically switchgrass, have high biomass yield potential, can be cultivated on agricultural or marginal land, and are often associated with ecological services (McLaughlin and Walsh, 1998; Parrish and Fike, 2005). Compared to annual crops like corn, perennial grasses can reduce soil erosion (McLaughlin and Walsh, 1998), provide greater nutrient capture and utilization, increase carbon storage and soil organic matter (Frank et al., 2004), and reduce loss of soil nutrients and improve soil fertility (McLaughlin and Kszos, 2004; Sanderson and Adler, 2008; Tilman et al., 2006). While perennial grasses show great potential as bioenergy crops (Casler et al., 2007; Lee and Boe, 2005), there is debate about how they should be cultivated to maximize both biomass yield and ecological benefits. Proposed perennial bioenergy cropping systems include grass monocultures (Adler et al., 2006; Lee and Boe, 2005; Lemus et al., 2002), low diversity polycultures (Picasso et al., 2008; Tracy and Sanderson, 2004), or high diversity polycultures (Tilman et al., 2006). These cropping systems include a range of species diversity and require different management strategies. The greatest distinction between these cropping systems is the proposed level of species richness and desired botanical composition. Ecologists have traditionally advocated for high diversity mixtures for biodiversity conservation while agronomists have advocated for low diversity mixtures or monocultures to maximize biomass yield (Picasso et al., 2008; Russelle et al., 2006). According to Tilman et al. (2006), polycultures of native grasses, legumes, and forbs

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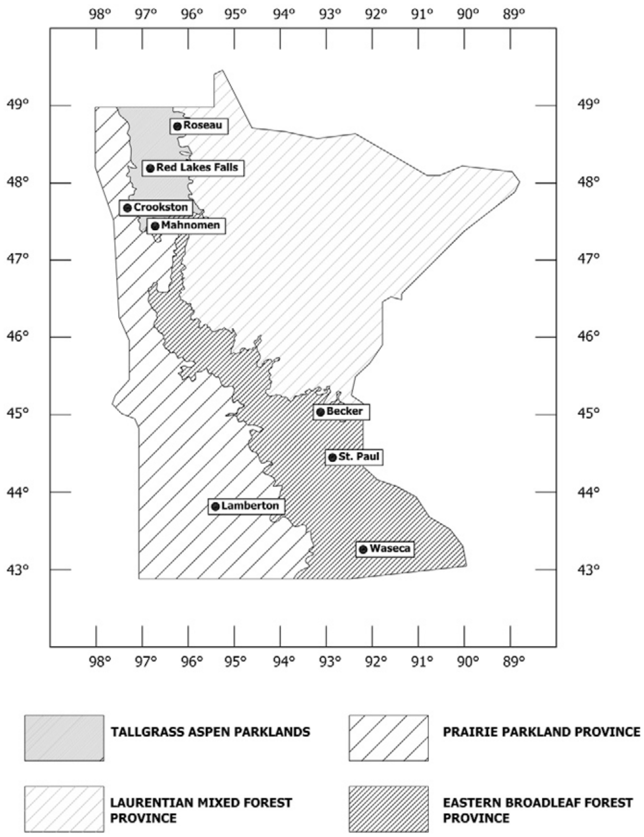

Fig. 1. Location of research sites used to evaluate the establishment and initial biomass productivity of different perennial bioenergy cropping systems.

produced up to 268% more ethanol per unit area than monocultures when managed with low agricultural inputs. This assertion is based on a long-term experiment at Cedar Creek Ecosystem Science Reserve, in central Minnesota, where research plots were managed as a restored grassland ecosystem (i.e., annual burning and hand weeding). At this location, soil nutrients are limited and switchgrass monocultures performed poorly. Meanwhile, switchgrass monocultures and low diversity mixtures have been highly productive in other experiments (Adler et al., 2006; Lee and Boe, 2005; Schmer et al., 2008). For example, Schmer et al. (2008) showed that agriculturally managed switchgrass monocultures had 93% greater ethanol yield than low-input, high-diversity polycultures and 400% greater ethanol yield than low input switchgrass monocultures.

The highest yielding perennial bioenergy cropping system may therefore be somewhere between randomly assembled high diversity polycultures and monocultures of grasses. High biomass yields may be achieved using a strategic combination of a few species with specific contributions to biomass or energy yield (Picasso et al., 2008; Tracy and Sanderson, 2004). Minns et al. (2001) suggested that legumes may have a "keystone effect" on overall biomass productivity of polycultures due to their ability to fix atmospheric N_2, which is subsequently used by other species and results in greater overall aboveground biomass. This implies that binary mixtures of grasses and legumes may have comparable yields to treatments with higher species richness. However, Reich et al. (2004) observed a broader positive effect of increased functional group and species richness on native polyculture productivity, even in mixtures that did not include legumes. Functional groups are plants with similar physiological and morphological characteristics, which dictate their resource requirements, seasonality of growth, and life history (Tilman et al., 1997). Mixing species with different life cycles and nutrient requirements reduces interspecies competition while maintaining overall biomass productivity and stability (Tilman and Downing, 1994), with the individual growth characteristics of species in a mixture as important as the number of species (Hooper and Vitousek, 1997; Loreau et al., 2001).

Regardless of the species combination, the feasibility of establishing and maintaining diverse polycultures in an agricultural setting remains unclear. High levels of soil nutrients in agricultural fields may result in polycultures dominated by a few highly competitive species (Pywell et al., 2003). The dominance of a particular subset of species may preclude the establishment of high diversity mixtures. According to Pywell et al. (2003), high residual soil fertility associated with repeated fertilizer applications favors grasses and limits the establishment of high diversity mixtures, consistent with results for long-term perennial grass polycultures, even under rising carbon dioxide levels (Reich, 2009). Moreover, high nutrient levels provide favorable conditions for competitive annual and perennial weeds, which directly compete with native perennials (Pywell et al., 2003). To enhance widespread adoption, we must evaluate the impact of increasing species richness in bioenergy cropping systems on soils commonly used in agriculturally managed systems.

Research is needed to design productive native herbaceous bioenergycropping systems in diverse soils and climates. Our objective was to determine the effect of species richness and composition on establishment and initial biomass production of native plant polycultures across diverse eco-regions in Minnesota.

MATERIALS AND METHODS

The experiment was conducted at eight sites located within multiple ecological regions of Minnesota (Fig. 1). The experiment was established at the University of Minnesota Experiment Station in St. Paul, the Southern Research and Outreach Center in Waseca, the Sand Plain Research Farm in Becker, the Southwest Research and Outreach Center in Lamberton, the Northwest Research and Outreach Center in Crookston, the Magnusson Research Farm in Roseau, and on private agricultural land in Red Lake Falls and Mahnomen. Research sites located north of 46°N hereafter are referred to as northern locations and research sites located south of 46°N are referred to as southern locations. Northern and southern sites are demarcated according to differences in temperature and precipitation, which affect crop growth. The eight research sites represent a wide precipitation and temperature gradient and encompass a variety of soil types (Table 1).

Soil samples were collected to a 30.5-cm depth before site preparation and analyzed for N, P, K, S, total carbon, organic matter, and pH at all sites (Brown, 1998) (Table 2). No fertilizer was applied during the study. The previous crop was corn at St. Paul, Waseca, and Lamberton, rye (*Secale cereale* L.) at Becker, soybean [*Glycine max* (L.) Merr.] at Roseau, and spring wheat (*Triticum aestivum* L.) at Red Lake Falls, Mahnomen, and Crookston.

The experimental design was a randomized complete block with six replications per location (288 plots). Twelve treatments with varying levels of species richness (1, 4, 8, 12, or 24 species)

Table 1. Location, major soil type, soil description, precipitation, and temperature at eight experimental sites used to evaluate the establishment and initial biomass productivity of different perennial bioenergy cropping systems.

Location	Soil type	Soil description	Seeding year precipitation May– July	Seeding year precipitation Aug.–Oct.	First harvest year precipitation May– July	First harvest year precipitation Aug.–Oct.	30-Year average precipitation May– July	30-Year average precipitation Aug.–Oct.	30-Year average temperature May– July	30-Year average temperature Aug.–Oct.
			mm						°C	
St. Paul	Waukegan	Fine-silty over sandy, mixed, superactive, mesic Typic Hapludoll	224.0	280.9	181.6	440.9	333.2	256.0	19.5	16.0
Waseca	Webster	Fine-loamy, mixed, superactive, mesic Typic Endoaquolls	296.9	257.6	250.4	535.9	320.5	262.4	18.8	15.0
Lamberton	Normania	Fine-loamy, mixed, superactive, mesic Aquic Hapludolls	207.0	197.4	136.4	358.4	272.0	200.4	19.2	15.1
Becker	Hubbard	Sandy, mixed, frigid Entic Haploboroll	202.2	245.1	157.5	366.0	300.0	252.5	18.6	14.8
Roseau	Bearden Colvin Fargo	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls Fine-silty, mixed, superactive, frigid Typic Calciaquolls Fine, smectitic, frigid Typic Epiaquerts	293.9	200.7	na†	na	235.7	181.6	16.1	11.9
Red Lake Falls	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	313.4	222.5	183.9	315.0	249.7	207.8	17.1	12.8
Mahnomen	Barnes Langhei	Fine-loamy, mixed, superactive, frigid Calcic Hapludolls Fine-loamy, mixed, superactive, frigid Typic Eutrudepts	289.8	199.6	235.5	396.0	255.5	195.8	16.6	12.6
Crookston	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	265.7	220.5	181.4	311.7	230.4	178.3	17.6	13.5

† na: data not available.

were established with selected native tallgrass prairie species. Species included in the study were nonrandomly selected according to natural abundance in native grasslands, aboveground biomass yield potential, N_2 fixation capability, and availability of seed and inoculants. Plant functional groups in this experiment were grasses, legumes, and nonleguminous forbs. The grasses selected for the study are highly productive and dominate in natural tallgrass prairie ecosystems (Jacobson, 2005; Knapp et al., 1998). Plant species varied slightly between northern and southern locations and represented the most dominat species in each region (Table 3). All seeds were grown or collected in Minnesota and were purchased from Kaste Inc. (Fertile, MN), Prairie Moon Nursery (Winona, MN), Feder Prairie Seed Company (Blue Earth, MN) or Carlson Prairie Seed Farm (Halma, MN).

Plots were seeded in June 2006 in southern Minnesota and June 2007 in northern Minnesota. At the southern locations, weed populations were suppressed by applying Vapam (Sodium N-methyldithiocarbamate) 1 mo before planting at 84 kg ha^{-1} and incorporated using a spring tooth harrow. The northern research sites did not receive a Vapam preplanting treatment. Legume seeds were scarified using a mechanical compressed air/sandpaper scarifier. Seed was inoculated before planting using the inoculation recommendations of Tlusty et al. (2004) with liquid cultures grown 48 h in accelerated BYMA broth cultures (Graham, 1963).

Seed mixes were manually broadcast into individual plots (9 m^2) that were separated by 1-m alleys. The entire experimental area was 779 m^2 and surrounded by 3-m borders along the exterior. The alleys and borders were planted with a mixture of switchgrass, big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* L.), and Canada wild rye (*Elymus canadensis* L.) to control edge effects. Each plot received 108 g of seed (12 g m^{-2}) and was never reseeded regardless of establishment success. Seed mass was divided equally among the number of species in each treatment. We planted by seed mass instead of pure live seed because we wanted to provide about the same amount of total seed energy per plot even though the procedure resulted in variable numbers of seed per species. This approach has been previously used in ecological studies (Janneke et al., 2004; Reich et al., 2004). Plots were hand raked before and after seeding.

During June–August 2006, the establishment year, southern locations were watered twice per week to avoid desiccation of seedlings. During 2007 and 2008, no irrigation was applied. Between 2006 and 2008, plots were hand weeded only when weeds developed a canopy and excluded light penetration for developing seedlings. Otherwise, annual weeds were tolerated and considered part of the field environment. Because of excessive weed populations, the St. Paul experiment was mowed at 2-wk intervals from

Table 2. Characterization of top 30.5 cm of soil during seeding year, before initiation of experiments, at St. Paul, Waseca, Lamberton, Becker, Roseau, Red Lake Falls, Mahnomen, and Crookston.

Location	pH	OM†	TOC	TKN	Nitrate	S	Bray extractable P	K
		g kg^{-1}			kg ha^{-1}		mg kg^{-1}	
St. Paul	6.9	44	28	2.6	24	34	127	373
Waseca	6.5	43	23	2.2	30	31	18	189
Lamberton	5.7	36	22	2.3	14	18	71	601
Becker	6.6	14	9	0.7	8	11	30	108
Roseau	7.2	16	11	1.1	7	24	18	121
Red Lake Falls	7.2	21	18	1.6	4	16	20	115
Mahnomen	7.7	33	25	2.1	8	19	14	162
Crookston	8.3	27	na‡	na	40	na	14	145

† OM: organic matter, TOC: total organic carbon, TKN: total Kjeldahl nitrogen.
‡ na: data not available.

Table 3. Native species and treatments used to evaluate the establishment and initial biomass productivity of perennial bioenergy cropping systems. Native species are organized according to their functional group and a list of species combinations for each treatment. Species denoted with ‡ where substituted at northern locations. Experimental plots were planted in June 2006 at four southern locations and June 2007 at four northern locations.

Native grasses	Native legumes	Native forbs
A- Switchgrass, *Panicum virgatum* L.	I- Canada milkvetch, *Astragalus canadensis* L.	Q- Butterfly milkweed, *Asclepias tuberosa* L. or Purple coneflower, *Echinacea purpurea* L.†
B- Big bluestem, *Andropogon gerardii* Vitman	J- Wild blue indigo, *Baptisia australis* L. or Showy tick trefoil, *Desmodium canadense* L.†	R- Maximillian sunflower, *Helianthus maximiliani* Schrad.
C- Indiangrass, *Sorghastrum nutans* L.	K- Purple prairie clover, *Dalea purpurea* Vent.	S- Stiff goldenrod, *Solidago rigida* L.
D- Canada wild rye, *Elymus canadensis* L. or Virginia wild rye, *Elymus virginicus* L.†	L-Lead plant, *Amorpha canescens* Pursh	T- Yellow coneflower, *Ratibida pinnata* (Vent.) Barnhart
E- Little bluestem, *Schizachyrium scoparium* Michx.	M- Perennial lupine, *Lupinus perennis* L. or American licorice, *Glycyrrhiza lepidota* Pursh†	U- Rough blazing star, *Liatris aspera* Michx. or Northern bedstraw, *Galium boreale* L.†
F- Slender wheatgrass, *Elymus trachycaulus* Link	N- Partridge pea, *Chamaecrista fasciculate* Michx. or Pale pea, *Lathyrus ochroleucus* Hook.†	V- Wild bergamot, *Monarda fistulosa* L.
G- Sideoats grama, *Bouteloua curtipendula* Michx.	O- Showy tick trefoil, *Desmodium canadense* L. or White prairie clover, *Dalea candida* Michx.†	W- Cup plant, *Silphium perfoliatum* L. or Black-eyed Susan, *Rudbeckia hirta* L.†
H- Virginia wild rye, *Elymus virginicus* L. or Canada wild rye, *Elymus canadensis* L.†	P- Roundheaded bushclover, *Lespedeza capitata* Michx. or American vetch, *Vicia Americana* Muhl.†	X- Golden Alexander, *Zizia aurea* L.

Treatments

1 Switchgrass monoculture	7 Forb polyculture (Q, R, S, T)
2 Big bluestem monoculture	8 Grass + Legume polyculture (A, B, C, D, I, J, K, L)
3 Indiangrass monoculture	9 Grass + Forb polyculture (A, B, C, D, Q, R, S, T)
4 Wild rye monoculture‡	10 Legume + Forb polyculture (I, J, K, L, Q, R, S, T)
5 Grass polyculture (A, B, C, D)	11 Grasses + Legumes + Forbs (A, B, C, D, I, J, K, L, Q, R, S, T)
6 Legume polyculture (I, J, K, L)	12 High Diversity polyculture (A through X)

† Denotes species that were substituted at northern locations.

‡ Wild rye monoculture at southern locations refers to Canada wild rye and wild rye monoculture at northern locations refers to Virginia wild rye.

mid-July to September 2006 to a height of 15 cm and the mowed herbage residue was removed from the plots.

Detailed measurements and data collection started 1 yr after seeding. The first year of data collection (1 yr after seeding) is hereafter referred to as the first harvest year. Plant populations and percent vegetative cover of seeded natives and volunteer weeds were determined during late July in a 1.0 by 0.5 m quadrants placed in a representative area of each plot, while a 30-cm border from the edge was maintained. Due to the height variation of the plant material and the fact that taller plants covered a larger portion of the plot with shorter species intermixed, it was challenging to accurately assign vegetative cover values to individual forb and legume species (Kennedy and Addison, 1987; Marten, 1964). Therefore, populations of legumes and forbs were determined by counting the number of seedlings per 0.5 m^2. Species establishment was defined by the presence of a species at a single location and species richness refers to the number of different species present at a single location. Vegetative cover was measured for grass species in monocultures and mixtures. Because, native grass seedlings have similar morphological features during the establishment phase, individual grass species within mixtures were not assessed, but estimates for total vegetative cover of grass were recorded. Estimates for the vegetative cover of grasses were made by evaluating both the upper and lower grass canopy and averaging the vegetative cover in each canopy. In addition to grass species, estimates of weed populations were also recorded, but not reported.

The Berger–Parker index (Berger and Parker, 1970) was used to measure the dominant grass, legume, or nonleguminous forbs present in each polyculture during peak productivity and harvest. The Berger–Parker index is a diversity index that identifies dominant species according to their proportional abundance within a mixture. We define dominance, d, according to the proportion of aboveground biomass of a single species relative to the total aboveground biomass yield of the mixture, $d = N_{\max}/N$ (Magurran, 2004). In this equation N_{max} is defined as the aboveground biomass of the most abundant species.

After a frost (0°C), the biomass yield of all plots was determined by harvesting a 1.0 by 0.5 m area to a height of 10 cm. The remaining biomass was then removed with a flail-type harvester. The harvest dates ranged from 9 to 29 Oct. 2007 at southern locations and from 18 to 25 Oct. 2008 at northern locations. Harvested samples were weighed in the field, separated into component species in the laboratory, dried in an oven at 60°C for 48 h, and reweighed. Individual grasses within mixtures were not separated because of difficulty in identification, and a single weight for grasses was recorded. Total annual biomass yield for each plot was calculated by summing the dry weight of individual species present in the mixture.

STATISTICAL ANALYSIS

Differences in total biomass yield among treatments were determined using a two-way ANOVA with species mixture as the main effect. Likewise, differences in plant populations among treatments were analyzed using ANOVA with square root transformation of data while vegetative cover data was transformed using arcsine of the square root to homogenize variances. If the interaction between species mixture and location was significant ($P < 0.05$) then we analyzed each location separately. Least significance differences were used to separate means when F tests were statistically significant ($P < 0.05$).

We used orthogonal contrasts to test the linear effects of species richness on aboveground biomass yield. Additionally, we used orthogonal contrasts to compare biomass production between the highest yielding monoculture treatment and the highest yielding polyculture with 4, 8, 12, and 24 species at each location. We adjusted the critical α value using the Holm's test. The Holm's test uses a stepwise procedure to examine the ordered set of null hypotheses, beginning with the smallest *p* value, and continuing until it fails to reject the null hypothesis (Oehlert, 2000). When the interaction was significant, we calculated the regression coefficients and standard errors. Regression analysis was used to determine the relationship of vegetative grass cover in July and aboveground biomass yield in the fall. Vegetative cover and biomass yield of grasses in monocultures and polycultures were analyzed separately in this analysis. We calculated regression coefficients, standard errors and *t* statistics. All data analysis was conducted using R statistical software (R Development Core Team, 2010).

RESULTS

Establishment

Overall, 22 of the 24 species seeded were present at one or more locations during plant population surveys in July of the year following seeding. We have not observed cup plant (*Silphium perfoliatum* L.) or lead plant (*Amorpha canescens* Pursh) at southern locations and stiff goldenrod (*Solidago rigida* L.) or American licorice (*Glycyrrhiza lepidota* Pursh) at northern locations. Partridge pea (*Chamaecrista fasciculata* Michx.) established at southern locations during the 2006 growing season but was not present in 2007, although it is a self-seeding annual.

As expected, the number of species established in a polyculture treatment increased linearly as the number of species seeded increased ($R^2 = 0.79$ at St. Paul and R^2 of 0.90–0.98 at other locations). Thus, the seeding of high diversity polycultures consistently produced higher species richness than the seeding of lower diversity polycultures and seeded species richness was a good estimator of observed species richness.

Despite this strong association, species establishment was unique at each location and species richness, for a given initial planting, varied between the eight sites (Tables 4 and 5). In general, higher species richness was observed at northern locations with lower species richness at southern locations. However, due to the treatment × location interaction ($P < 0.001$), we cannot compare species richness across locations and must evaluate each location separately.

Although we seeded species on a mass basis that resulted in unequal numbers of viable seeds per m^2, variable seeding rates was not consistently related to establishment likely because small seeded species lacked seedling vigor and competitiveness compared to larger seeded species. For example, Maximilian sunflower is a larger seeded species thus there were fewer seeds planted, but in both southern and northern sites, it was consistently a dominant species (Tables 4 and 5). Additionally, showy tick trefoil and purple coneflower (treatments 8 and 9 in northern Minnesota) had fewer seeds planted but high seedling density. On the other hand, both stiff goldenrod and lead plant have very small seeds and thus were seeded at high rates; however, their seedling densities were very low and they were never dominant. As a result, in a regression analysis (data not shown) we found no significant correlation ($P > 0.05$) between species seeding rates and establishment.

Botanical Composition

We used two methods to describe aspects of botanical composition. We conducted plant population surveys to determine seedling density of legumes and nonleguminous forbs, while vegetative cover estimates were assigned to grass species in monocultures and polycultures.

Legume and Nonleguminous Forbs

Number of individual seedlings present in July varied among the plant functional groups (Tables 4 and 5). Overall, legumes had much smaller populations than nonleguminous forbs ($P < 0.001$). Canada milkvetch established the largest populations of legumes at all locations, while wild blue indigo (*Baptisia australis* L.) and lead plant had the smallest populations in southern and northern locations, respectively. Two exceptions were at Waseca and Red Lake Falls, where purple prairie clover (*Dalea purpurea* Vent.) and showy tick trefoil (*Desmodium canadense* L.) had the largest populations of legumes, respectively. Maximilian sunflower established the largest populations of any nonleguminous forb at all locations except St. Paul and Lamberton where yellow coneflower [*Ratibida pinnata* (Vent.) Barnhart] predominated. At Lamberton and Becker, Maximilian sunflower seedlings were four times as abundant as any other nonleguminous forb in the forb polyculture. At all northern locations except Mahnomen, Maximilian sunflower seedlings were three times as abundant as any other nonleguminous forb in all treatments that included the forb polyculture.

According to the Berger–Parker dominance index, the most common dominant species during July were Canada milkvetch, yellow coneflower, and Maximilian sunflower (Tables 4 and 5). On average, the dominant species accounted for 71% of seedlings present at southern locations and 53% of seedlings present at northern locations during the growing season. Botanical composition and species dominance were similar in the plant population survey in July and at fall biomass harvest, however, species richness was greater during July. This was because some species had fully senesced before harvesting and did not contribute to biomass yield.

Grass Monocultures and Polycultures

The amount of vegetative cover present in grass monocultures varied among locations during the first harvest year (Table 6). At Lamberton, all grass monocultures had vegetative cover >80% (±4%). Grass cover was more variable at other sites. At Waseca, St. Paul and Roseau, Canada wild rye was the predominant grass and had 86, 88, and 69% cover, respectively. At Crookston and Mahnomen, either big bluestem or switchgrass had the greatest cover. Indiangrass had similar cover as big bluestem or switchgrass at Becker, Roseau, Crookston, and Red Lake Falls.

Grasses were cultivated in a multispecies grass polyculture and in polycultures with legumes and nonleguminous forbs. We did not directly measure the vegetative cover of individual grasses within the grass polyculture (four grasses combined) because of the difficulty in quantifying differences between juvenile species. However, we visually estimated that either wild rye or switchgrass dominated mixtures at each location during plant population surveys, even at sites where big bluestem had the greatest cover in monocultures.

Herbage Biomass Yield

Location Effects

Biomass yields in the year after seeding varied greatly with location and treatment (Table 7). Average biomass yields in

Table 4. Populations of legumes and nonleguminous forbs 1 yr after planting at four southern Minnesota locations. Dominance was calculated as the maximum proportion of seedlings of a single species relative to the total number of seedlings established in the mixture.

Species (Treatment no.)	Planting rate	Location			
		St. Paul	Waseca	Lamberton	Becker
	seeds m^{-2}	seedlings m^{-2}			
Legume polyculture (no. 6)					
Wild blue indigo	159	0	15	1	6
Purple prairie clover	1587	0	113†	2	28
Canada milkvetch	1799	15†	52	150†	52†
Lead Plant	1693	0	0	0	0
Total		15	180	153	86
Dominance†		1	0.63	0.98	0.6
Forb polyculture (no. 7)					
Maximilian sunflower	1376	8	93	222†	266†
Stiff goldenrod	4339	8	2	0	1
Yellow coneflower	3175	150†	114†	18	57
Butterfly milkweed	455	7	23	4	40
Total		173	232	244	364
Dominance†		0.87	0.49	0.91	0.73
Grass + Legume polyculture (no. 8)					
Wild blue indigo	79	0	9	0	1
Purple prairie clover	794	0	65†	2	5
Canada milkvetch	899	3†	35	67†	14†
Lead Plant	847	0	0	0	0
Total		3	109	69	20
Dominance†		1	0.6	0.97	0.7
Grass + Forb polyculture (no. 9)					
Maximilian sunflower	688	9	65	149†	142†
Stiff goldenrod	2169	6	2	0	0
Yellow coneflower	1587	142†	76†	18	28
Butterfly milkweed	228	1	16	8	12
Total		158	159	175	182
Dominance†		0.9	0.48	0.85	0.78
Legume + Forb polyculture (no. 10)					
Wild blue indigo	79	0	5	0	1
Purple prairie clover	794	0	40	0	5
Canada milkvetch	899	1	24	12	10
Lead Plant	847	0	0	0	0
Maximilian sunflower	688	11	108†	181†	185†
Stiff goldenrod	2169	6	1	0	0
Yellow coneflower	1587	152†	74	11	60
Butterfly milkweed	228	5	14	4	27
Total		175	266	208	288
Dominance†		0.87	0.41	0.87	
Grasses + Legumes + Forbs (no. 11)					
Wild blue indigo	53	0	4	2	0
Purple prairie clover	529	0	49	0	0
Canada milkvetch	600	0	23	9	6
Lead Plant	564	0	0	0	0
Maximilian sunflower	459	8	55	187†	124†
Stiff goldenrod	1446	1	1	0	0
Yellow coneflower	1058	78†	73†	16	44
Butterfly milkweed	152	7	13	4	10
Total		94	218	218	184
Dominance†		0.83	0.33	0.86	0.67
High Diverstiy Polyculture (no. 12)					
Wild blue indigo	26	0	3	1	0
Purple prairie clover	265	0	29	2	0
Canada milkvetch	300	0	19	15	0
Lead plant	282	0	0	0	0
Maximilian sunflower	229	1	25	83†	72†
Stiff goldenrod	723	0	1	0	0
Yellow coneflower	529	26†	33†	18	15
Butterfly milkweed	76	2	4	5	4
Perennial lupine	19	0	7	0	2
Showy tick trefoil	97	0	9	6	25
Round headed bushclover	141	0	13	7	3
Partridge pea	48	0	0	0	0
Cup plant	25	0	0	0	0
Wild bergamot	1235	19	16	9	23
Rough blazing star	282	0	0	0	4
Golden Alexander	194	0	3	0	4
Total		48	161	147	153
Dominance†		0.54	0.21	0.56	0.47
LSD (0.05)‡		6	2	3	5
Species richness§		4	12	9	9

† Indicates dominant species in each treatment.

‡ LSD (0.05) for comparing differences between total seedlings per treatment.

§ Species richness refers to the number of different species that established at a single location.

Table 5. Populations of legumes and nonleguminous forbs 1 yr after planting at four northern Minnesota locations. Dominance was calculated as the maximum proportion of seedlings of a single species relative to the total number of seedlings established in the mixture.

Species (Treatment no.)	Planting rate	Location			
		Roseau	Red Lake Falls	Mahnomen	Crookston
	seeds m^{-2}	seedlings m^{-2}			
Legume polyculture (no. 6)					
Showy tick trefoil	582	28	58†	73	32
Purple prairie clover	1587	53	38	24	16
Canada milkvetch	1799	58†	57	82†	82†
Lead plant	1693	0	0	6	0
Total		139	153	185	130
Dominance†		0.42	0.4	0.44	0.63
Forb polyculture (no. 7)					
Stiff goldenrod	4339	0	0	0	0
Maximilian sunflower	1376	204†	237†	140†	280†
Yellow coneflower	3175	34	38	57	19
Purple coneflower	698	39	45	80	70
Total		277	321	277	369
Dominance†		0.74	0.74	0.51	0.76
Grass + Legume polyculture (no. 8)					
Showy tick trefoil	291	28	34†	35†	31
Purple prairie clover	794	49†	5	9	20
Canada milkvetch	899	46	26	33	37†
Lead plant	847	0	0	4	4
Total		122	65	81	92
Dominance†		0.4	0.52	0.43	0.4
Grass + Forb polyculture (no. 9)					
Stiff goldenrod	2169	0	0	0	0
Maximilian sunflower	688	196†	192†	96†	282†
Yellow coneflower	1587	39	36	28	28
Purple coneflower	349	50	51	30	51
Total		285	279	155	361
Dominance†		0.69	0.69	0.62	0.78
Legume + Forb polyculture (no. 10)					
Showy tick trefoil	291	23	42	48	15
Purple prairie clover	794	21	5	17	2
Canada milkvetch	899	28	29	41	17
Lead plant	847	0	0	12	0
Stiff goldenrod	2169	0	0	0	0
Maximilian sunflower	688	172†	199†	94†	278†
Yellow coneflower	1587	31	36	41	22
Purple coneflower	349	42	33	52	25
Total		317	343	306	359
Dominance†		0.54	0.58	0.31	0.77
Grasses + Legumes + Forbs (no. 11)					
Showy tick trefoil	194	30	29	35	21
Purple prairie clover	529	43	11	14	1
Canada milkvetch	600	30	29	33	22
Lead plant	564	0	0	8	0
Stiff goldenrod	1446	0	0	0	0
Maximilian sunflower	459	98†	143†	78†	256†
Yellow coneflower	1058	29	33	26	19
Purple coneflower	233	31	29	27	26
Total		260	273	222	345
Dominance†		0.38	0.52	0.35	0.74
High Diversity Polyculture (no. 12)					
Showy tick trefoil	97	8	15	20	10
Purple prairie clover	265	25	10	19	2
Canada milkvetch	300	9	11	26	11
Lead plant	282	0	0	0	0
American licorice	69	0	0	0	0
Pale pea	no data	3	0	4	0
White prairie clover	335	14	11	18	4
American vetch	no data	6	2	5	1
Stiff goldenrod	723	0	0	0	0
Maximilian sunflower	229	41	68	46	147†
Yellow coneflower	529	7	11	19	17
Purple coneflower	116	6	16	21	15
Northern bedstraw	1235	5	2	13	1
Wild bergamot	1235	12	20	11	10
Black-eyed Susan	1623	78†	80†	88†	26
Golden Alexander	194	20	8	14	7
Total		235	252	304	251
Dominance†		0.33	0.32	0.29	0.59
LSD (0.05)‡		5	4	2	4
Species richness§		13	12	13	12

† Indicates dominant species in each treatment.

‡ LSD (0.05) for comparing differences between total seedlings per treatment.

§ Species richness refers to the number of different species that established at a single location.

Table 6. The amount of vegetative cover present during the first harvest year in grass monocultures at the eight experimental sites used to evaluate the establishment and initial biomass productivity of different perennial bio-energy cropping systems.

Grass species	Location							
	St. Paul	Waseca	Lamberton	Becker	Roseau	Red Lake Falls	Mahnomen	Crookston
	%							
Switchgrass	6	78	98	76	57	88	88	67
Big bluestem	14	79	95	88	46	90	89	89
Indiangrass	26	47	83	83	58	93	72	72
Wild rye†	88	86	93	37	69	71	56	64
Average	34	73	92	71	58	86	76	73
LSD (0.05)‡	5	6	1	4	15	9	6	7

† Wild rye monoculture at southern locations refers to Canada wild rye and wild rye monoculture at northern locations refers to Virginia wild rye.

‡ LSD (0.05) for comparing differences between vegetative cover per treatment.

southern Minnesota ranged from 1.2 Mg ha^{-1} at Becker to 6.0 Mg ha^{-1} at Lamberton, and in northern Minnesota from 4.4 Mg ha^{-1} at Mahnomen to 5.5 Mg ha^{-1} at Crookston. Low average yields at Becker were related to the loamy sand soil, which has a low water holding capacity and typically requires irrigation for average crop yields. In addition, there was below average rainfall during the first harvest year. Low average yields at St. Paul were related to strong competition from annual weeds and below average rainfall at critical times during both the seeding and first harvest year. The Normania clay loam at Lamberton contained high levels of P and K and has a high water holding capacity that supported abundant biomass production. The relatively higher yields at the northern locations were somewhat surprising because of the shorter growing season. We explored the effect of environment on biomass yield but did not observe a consistent relationship between yield and precipitation or soil characteristics during the establishment phase. As expected, because of these diverse environments and the unique resource requirements of dominant species, a location × treatment interaction occurred. Therefore, we analyze the aboveground biomass yield at each location separately.

Monocultures vs. Polycultures

The highest yielding grass monoculture at each location had equal or greater biomass yields than the highest yielding polyculture. The wild rye monoculture was the highest yielding monoculture at St. Paul, Lamberton, Roseau, Red Lake Falls, and Crookston, while the switchgrass monoculture had the highest yields at Waseca, Becker, and Mahnomen. The grass polyculture (four grass species combined) had a similar biomass yield as the highest yielding grass monoculture at five of the eight sites (Lamberton, Becker, Red Lake Falls, Mahnomen, and Crookston).

The species composition of most mixtures differed among locations and relative yields of polycultures changed. At Lamberton, the grass polyculture, dominated by Canada wild rye, had greater biomass yield than all other polycultures. In contrast, biomass yield of the grass polyculture at St. Paul, Waseca, and Mahnomen, was similar to those of the forb polyculture, grass–legume polyculture, and grass–legume–forb polyculture. The 24-species polyculture (treatment 12) was the most productive polyculture only at St. Paul. At Becker, the grass polyculture and the grass–legume polycultures had comparable yields and were higher yielding than more diverse mixtures containing grasses, forbs, and legumes. The forb polyculture and the legume–forb polyculture were primarily composed of Maximilian sunflower and were among the lowest yielding mixtures at Becker. The legume polyculture was consistently among the lowest yielding treatment at each location.

Species Richness

To determine the effect of species richness on biomass yield, we averaged biomass yields across treatments with the same level of species richness (1, 4, 8, 12, and 24). Effects were inconsistent over locations. At all northern locations and at St. Paul in the south, mean aboveground biomass yield increased when species richness increased from one to eight species (Table 8). Increasing species richness from 8 to 12 to 24 species did not increase biomass yield except at St. Paul and Lamberton. The 24-species polyculture (treatment 12) was the most productive polyculture at St. Paul, although not different than the grass–legume or grass–forb mixtures. At Lamberton, greater biomass yield for the 12 species (grass–legume–forb mixture) compared to the 24 species mixture can be attributed to the performance of the dominant species, Maximilian sunflower, which contributed on average 79% of the aboveground biomass yield (Table 9).

Table 7. Aboveground biomass yield during the first harvest year at St. Paul, Waseca, Lamberton, Becker, Roseau, Mahnomen, Red Lake Falls, and Crookston.

Treatments	St. Paul	Waseca	Lamberton	Becker	Roseau	Red Lake Falls	Mahnomen	Crookston
	Mg ha^{-1}							
Switchgrass	0.2	6.1	8.5	2.1	2.3	3.7	5.6	4.1
Big Bluestem	0.0	1.4	3.6	0.7	0.7	2.2	2.8	1.5
Indiangrass	0.2	1.6	3.6	0.7	1.0	3.8	3.1	2.6
Wild rye	5.6	3.3	8.8	0.8	6.3	7.5	2.4	7.7
Grass polyculture (G)	3.2	3.8	9.1	2.1	3.4	6.4	5.0	8.0
Legume polyculture (L)	0.2	1.5	4.5	0.1	5.3	3.5	4.1	4.5
Forb polyculture (F)	2.3	4.3	6.5	0.5	6.2	4.1	3.6	6.6
G + L	3.7	4.1	4.6	2.1	7.2	8.9	5.5	6.4
G + F	3.6	3.4	6.2	1.3	4.9	4.2	4.7	6.1
L + F	2.3	3.6	5.0	0.7	7.2	4.6	5.2	6.4
G + L + F	2.4	4.4	6.9	1.5	5.6	4.7	5.6	6.3
High diversity mixture	4.6	3.8	4.7	1.3	6.8	6.8	4.8	6.2
Average	2.4	3.5	6.0	1.2	4.7	5.0	4.4	5.5
LSD (0.05)†	1.2	1.3	1.4	0.4	1.9	2.0	1.2	1.8

† LSD (0.05) for comparing differences between total biomass yield per treatment.

Table 8. Estimated Values (Estimate†) and significance value for orthogonal contrasts of biomass yield between treatments with different levels of species richness. Analysis was conducted at the eight locations used to evaluate the establishment and initial biomass productivity of different perennial bio-energy cropping systems.

Contrast		St. Paul		Waseca		Lamberton		Becker	
Species richness		Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
		Mg ha $^{-1}$							
1	4	0.4	ns‡	0.1	ns	0.6	ns	–0.2	ns
1	8	1.7	<0.001	0.6	ns	–0.8	ns	0.3	ns
1	12	0.9	NS	1.3	ns	0.8	ns	0.4	ns
1	24	3.0	<0.001	0.7	ns	–1.4	ns	0.3	ns
4	8	1.3	<0.001	0.5	ns	–1.4	<0.001	0.5	<0.001
4	12	0.5	ns	1.2	ns	0.2	ns	0.6	<0.001
4	24	2.7	0.001	0.6	ns	–2.0	<0.001	0.5	0.004
8	12	–0.8	ns	0.8	ns	1.6	0.005	0.1	ns
8	24	1.4	0.009	0.1	ns	–0.6	ns	–0.0	ns
12	24	2.1	0.001	–0.6	ns	–2.2	0.002	–0.1	ns

Contrast		Roseau		Red Lake Falls		Mahnomen		Crookston	
Species richness		Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
		Mg ha $^{-1}$							
1	4	2.4	<0.001	0.4	ns	0.7	ns	2.4	<0.001
1	8	3.9	<0.001	1.6	0.003	1.6	<0.001	2.3	<0.001
1	12	3.0	<0.001	0.4	ns	2.2	<0.001	2.3	0.002
1	24	4.2	<0.001	2.5	0.002	1.3	ns	2.2	0.003
4	8	1.5	0.007	1.3	ns	0.9	ns	–0.1	ns
4	12	0.6	<0.001	0.0	ns	1.4	0.005	–0.1	ns
4	24	1.9	ns	2.1	ns	0.5	ns	–0.2	ns
8	12	–0.9	ns	–1.3	ns	0.5	ns	0.0	ns
8	24	0.3	ns	0.8	ns	–0.4	ns	–0.1	ns
12	24	1.2	ns	2.1	ns	–0.9	ns	–0.1	ns

† Estimate represents the difference in biomass yield between levels of species richness. A positive estimate value indicates that treatments with greater species richness provided higher yields.
‡ ns: not significant at α = 0.05, when we apply the Holm's test (Oehlert, 2000).

This high level of dominance suggests that the presence of the dominant species probably had a greater effect on initial stand productivity than did species richness per se. According to the Berger–Parker dominance index, the species that dominated most plots were Canada milkvetch, Maximilian sunflower, or grasses (four grasses combined). On average, the dominant species accounted for 76% of the biomass yield at southern locations and 77% of the biomass yield at northern locations. This level of dominance indicates that during the establishment phase a single species provides the majority of aboveground biomass across all polycultures. Thus, the productivity of any polyculture was a function of one or two predominant species in the mixture.

DISCUSSION AND CONCLUSION

Establishment of specific native species in monocultures and in polycultures over diverse environments may be challenging because environmental conditions strongly influence establishment. For example, big bluestem monocultures had the greatest establishment at moisture-limited sites such as Becker and at sites with the least amount of precipitation during the seeding year. Meanwhile, Canada wild rye had the greatest vegetative cover at southern locations with the most early season (May–June) precipitation during both the seeding and first harvest year. The distribution of other species appears to be more influenced by soil characteristics. For example, yellow coneflower prefers well-drained clay and sandy soil with a pH between 6 and 7 (USDA NRCS National Plants Database, 2010). In our experiment, the largest populations of yellow coneflower were observed at St. Paul and Waseca, which have the preferred soil conditions. Thus, designing native plant mixtures that include species with contrasting habitat affinities would likely result in at least one species establishing well.

While some native species require specific environmental conditions to establish, other species grow well across a wide range of environments (Stevens et al., 2004; Weaver, 1968). Early successional species such as Canada milkvetch and Maximilian sunflower established vigorously and were initially dominant across all research sites. A common characteristic of these species is a thick and sturdy stem, which enables them to occupy the upper canopy and intercept light. Light is often the limiting factor in high diversity polycultures (Hautier et al., 2009; Kleijn, 2003), making an upright growth form advantageous. Additionally, both species have a perennial root crown and rhizomatous spreading system. This growth pattern enables them to quickly spread and form dense populations (USDA NRCS National Plants Database, 2010). These species provided high vegetative cover and biomass yield during the first harvest year. This early biomass production is critical in terms of economic profitability for landowners.

Based on ecological studies, we expect that early successional species that were dominant during the establishment phase of our experiment will contribute less biomass as stands mature and later successional species will become more dominant and provide greater biomass (Knapp et al., 1998). However, it is possible the dominance of early successional species in fertile agricultural fields may severely reduce the development of populations of other species and overtime aboveground biomass productivity may decline. For this reason, the growth characteristics of individual species and their competitive interactions must be considered when seeding low or high diversity bioenergy cropping systems. Reducing the seeding rate of early successional species may be one way to ensure the long-term stability and biomass productivity of perennial polycultures. If seeding rates are reduced, dominant species may be less suppressive allowing more species to coexist (Kleijn, 2003). To most accurately understand the succession of high diversity polycultures, botanical composition must be monitored and individual species should not be replaced after the initial planting. Ecological studies that have manipulated species

Table 9. Dominant species contributing to biomass yield, by treatment, 1 yr after seeding. The dominant species in the fall were identified as having the greatest aboveground biomass yield in a plot and the dominance index (Dom.) was calculated as the maximum proportion of biomass yield of a single species relative to the total biomass yield in the mixture.

Treatments†	St. Paul			Waseca			Lamberton			Becker		
	Species	Dom‡	SE§	Species¶	Dom	SE	Species	Dom	SE	Species	Dom	SE
Legume polyculture	C. milkvetch	1.00	0.00	C. milkvetch	0.99	0.00	C. milkvetch	1.00	0.00	C. milkvetch	1.00	0.00
Forb polyculture	Y. coneflower	0.92	0.03	Y. coneflower	0.49	0.13	Max. sunflower	0.95	0.02	Max. sunflower	0.97	0.01
Grass + Legume poly.	Grasses	1.00	0.00	Grasses	0.73	0.05	C. milkvetch	0.63	0.07	Grasses	1.00	0.00
Grass + Forb poly.	Y. coneflower	0.51	0.06	Grasses	0.42	0.08	Max. sunflower	0.85	0.01	Grasses	0.73	0.04
Legume + Forb poly.	Y. coneflower	0.90	0.03	Max. sunflower	0.58	0.06	Max. sunflower	0.89	0.02	Max. sunflower	0.89	0.04
Grass+Legume+Forb	Y. coneflower	0.46	0.06	Max. sunflower	0.37	0.09	Max. sunflower	0.80	0.07	Grasses	0.77	0.07
High Diversity poly.	Y. coneflower	0.91	0.04	Grasses	0.47	0.05	Max. sunflower	0.44	0.08	Grasses	0.74	0.05
	Roseau			**Red Lake Falls**			**Mahnomen**			**Crookston**		
Legume polyculture	C. milkvetch	1.00	0.00	C. milkvetch	0.96	0.02	C. milkvetch	0.94	0.01	C. milkvetch	0.99	0.00
Forb polyculture	Max. sunflower	1.00	0.00	Max. sunflower	0.97	0.01	Max. sunflower	0.96	0.01	Max. sunflower	0.99	0.00
Grass + Legume poly.	C. milkvetch	0.53	0.07	Grasses	0.89	0.03	Grasses	0.56	0.07	Grasses	0.70	0.09
Grass + Forb poly.	Max. sunflower	0.93	0.03	Max. sunflower	0.77	0.07	Max. sunflower	0.53	0.12	Max. sunflower	0.92	0.04
Legume + Forb poly.	Max. sunflower	0.88	0.04	Max. sunflower	0.67	0.11	Max. sunflower	0.72	0.05	Max. sunflower	0.99	0.00
Grass+Legume+Forb	Max. sunflower	0.79	0.05	Max. sunflower	0.50	0.09	Max. sunflower	0.57	0.08	Max. sunflower	0.93	0.02
High Diversity poly.	Black-eyed Susan	0.58	0.11	Y. coneflower	0.27	0.10	C. milkvetch	0.29	0.04	Max. sunflower	0.59	0.04

† Abbreviations of treatments: Grass, Grass Polyculture; Legume, Legume Polyculture; Forb, Forb Polyculture; High Diversity poly., High Diversity polyculture.

‡ Dominance scale is from 0–100%, where 100 means that all seedlings in the plot represent a single species.

§ Standard error (SE) is reported as the standard error of the mean across six replications.

¶ Abbreviations of species: C. milkvetch, Canada milkvetch; Y. coneflower, yellow coneflower; P. prairie clover, purple prairie clover; Max. sunflower, Maximilian sunflower; Grasses, switchgrass, big bluestem, Indiangrass, wild rye.

richness by manually establishing or replacing species in polycultures, after initial establishment (e.g.,Tilman et al., 1996), may provide results that are not useful for the application of perennial polycultures in agricultural settings.

The economic viability of native perennial bioenergy cropping systems is primarily dependent on yield and native plants are generally slow to reach full biomass productivity (Schmer et al., 2008). It is therefore important to determine the initial biomass production of different herbaceous plantings. In our experiment, a grass monoculture or grass–legume mixture provided the highest biomass yields and at most locations the highest yielding grass monoculture had equal or greater biomass yields than the highest yielding polyculture with 4, 8, 12, or 24 species. Our results concur with other research that found yields of diverse polycultures are often lower than the best yielding monoculture (Cardinale et al., 2006). However, predicting a priori which species will perform best at a site, given variations in weather might be difficult.

The productivity of the polycultures was a function of one or two predominant species in the mixture. Even in the highest diversity treatment, between 27 and 91% of the biomass came from a few predominant species. Therefore, increasing overall species richness may not be as important as combining certain species that are compatible in mixtures and have specific contributions to biomass or energy yield. Thus, strategically designed, low diversity polycultures may provide comparable biomass yields as randomly assembled high diversity polyculture, if species in the mixture are compatible and have high yield potential.

We conclude that during the establish phase the aboveground biomass yield and relative dominance of species is determined greatly by location. We anticipate that variables such as soil type, precipitation, number of growing degree days, and temperature will determine differences in biomass productivity over time. Many important questions remain about the use of perennial polycultures for biomass production. Central among these is to determine initial mixture composition and cultural practices that promote high long-term biomass yields in diverse environments and how biomass harvest affects soil fertility.

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