

ARTICLE

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Nitrogen fertilizer rate effects on yield and botanical components of summer annual forage mixtures

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Abstract

Summer annual grass-legume mixtures may provide supplemental grazing for livestock when cool-season pastures are less productive, but nitrogen fertilizer recommendations for these mixtures are not well established. Inputs to these systems are often high, so optimizing N fertilizer rates may increase appeal to producers. This study evaluated the effects of increasing botanical diversity and N fertilizer application on the yield and botanical composition of summer annual mixtures in four environments in Kentucky. Nitrogen fertilizer rates of 0, 56, 112, 168, and 224 kg N ha⁻¹ were applied to a sudangrass [*Sorghum bicolor* (L.) Moench × *Sorghum sudanese* (P.) Stapf] monoculture, a three-species mixture, and an 11-species mixture. In three out of four environments, sward biomass increased as N application increased (average of 15 kg dry matter (DM) ha⁻¹ increase per kg N ha⁻¹; $p < .05$). Mixture complexity had no effect on forage DM accumulation in three out of four environments (4,000, 5,830, and 7,280 kg DM ha⁻¹ averaged over mixture for three environments; $p > .05$). Mixtures were overwhelmingly dominated by grasses, resulting in low functional diversity. Legumes did not respond to N ($p > .05$), but their contribution to sward DM was <4%. Species compatibility should be a priority when utilizing multi-species mixtures. If a stronger legume component is desired, care must be taken to provide management that favors these species, such as reduced grass-seeding rates to limit competition, especially during initial establishment.

1 | INTRODUCTION

Summer annual forages have the potential for high production and nutritive value during the summer months when perennial cool-season pasture growth is limited by high temperatures (Moser & Hoveland, 1996). Tracy et al. (2010) found that summer annual pastures exhibited 61% more production and equal or greater nutritive value as compared to cool-season grass pastures during the summer months. However, annual pastures incur establishment costs every year, leading many

to conclude that the enterprise is a “breakeven proposition at best” (Ball et al., 2007).

Increasing yield potential and reducing production costs may increase attractiveness of summer annual forage systems. One way to accomplish these goals may be by planting multiple species together, a practice known as intercropping. Yield potential is often increased in intercropping systems through niche differentiation, whereby species with differing morphology and physiology utilize resources more efficiently than that of a single species (Tofinga et al., 1993).

Numerous studies have documented increased land equivalent ratios or economic advantages for intercropping as

Abbreviations: DM, dry matter.

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compared to monocropping, but degree of benefit is dependent upon resource availability and level of interspecies competition (Lithourgidis et al., 2011). Land equivalent ratios from 1.25 to 1.94 have been reported in various grass–legume intercropping systems (Bybee-Finley et al., 2016; Emuh, 2007; Fan et al., 2020; Naik et al., 2017; Osiru & Willey, 1972). A land equivalent ratio of 2.2 was even reported in a three-species corn (*Zea mays* L.)/common bean (*Phaseolus vulgaris* L.)/mung bean [*Vigna radiata* (L.) R. Wilczek] mixture harvested for grain (Takele et al., 2017). In regards to economic advantage, Naik et al. (2017) reported nearly 20% increase in benefit/cost ratio of a corn–legume intercrop, while Andrews (1972) reported 80% more economic return per land unit area in intercropping as compared to sorghum [*Sorghum bicolor* (L.) Moench] monoculture.

The inclusion of legumes into a system is often a driver of increased productivity (Huston et al., 2000). In perennial systems, legumes will fix and “share” N with grasses during the growing season via root exudates, litter decomposition, and/or redistribution via livestock manure and urine (Paynel & Cliquet, 2003; Whitehead, 2000). It is less clear as to what degree this happens in annual cropping systems, as results are often inconsistent (Layek et al., 2012).

Fujita et al. (1992) and Layek et al. (2018) summarized results from numerous studies regarding N transfer in annual systems. Some studies found that no significant N transfer took place when legumes were included into intercropping systems but noted that residues may provide a substantial supply of N to subsequent crops (Izaurrealde et al., 1992; Wahua & Miller, 1978). Other studies observed the non-legume component contained more than 50% N derived from neighboring legumes (Eaglesham et al., 1981; Fujita et al., 1990).

Due to the inconsistencies in N transfer, fertilization recommendations for summer annual grass–legume mixtures are not well established. Nitrogen fertilizer application in these systems more strongly affects the non-legume component and may even be detrimental to legume productivity, but still may result in greater productivity or economic viability of the system as a whole (Ezumah et al., 1987; Haruna et al., 2006). In a corn/cowpea [*Vigna unguiculata* (L.) Walp.] system, Ezumah and colleagues (1987) reported a 27% decrease in cowpea grain yield when fertilized with 120 kg N ha⁻¹ as compared to no N, but corn grain yield increased by 62%. The increase in corn yield more than compensated for the decrease in cowpea yield, resulting in a 50% increase in total grain yield. Similarly, in a sorghum/soybean intercropping system, Haruna and colleagues (2006) reported a reduction in soybean grain yield and an increase in sorghum grain yield when fertilized with 100 kg N ha⁻¹ vs. no N, leading to a 28% increase in gross economic margin for the intercrop. Alternatively, Chowdhury and Rosario (1992) observed the greatest land equivalent ratio of corn/mung bean grain intercropping with only 30 kg N ha⁻¹ as compared to rates up to 120 kg N ha⁻¹.

Core Ideas

- 224 kg N ha⁻¹ increased yield by 1.9 times over no N in three out of four environments.
- Mixtures did not outyield monocultures and were dominated by high-yielding species.
- Legumes are not competitive when planted with high seeding rates of summer annual grasses.
- Annual pastures should utilize compatible species to reduce competitive dominance.

Similar to other cropping systems, N response for annual grass–legume mixtures managed for forage are not consistent across species, geographic location, and climatic variables. This inconsistency leads to variability in N fertilizer recommendations, and presently, none are available for annual grass–legume mixtures managed for forage in Kentucky. The objective of the current experiment was to determine the impact of N fertilizer rates on total aboveground biomass production and individual species components of simple and complex mixtures of summer annual forages. It was hypothesized that more complex mixtures would result in greater biomass accumulation and exhibit reduced response to N fertilization as compared to grass monocultures or simple grass–legume mixtures.

2 | MATERIALS AND METHODS

2.1 | Site description

This experiment was conducted at Lexington, KY (38.128, –84.498), and Princeton, KY (37.101, –87.854), in 2018 and 2019. Soil series were a Maury silt loam (fine, mixed, active, mesic Typic Paleudalf) and a Zanesville silt loam (fine-silty, mixed, active, mesic Oxyaquic Fragiudalf) (Soil Survey Staff, 2019) in Lexington and Princeton, respectively. Previous land use in Princeton for both years and in Lexington 2019 was cool-season perennial pasture. Area in Lexington 2018 was previously cropped with graminoid species. Temperature and precipitation data for each site was obtained from on-farm weather stations in the Kentucky Mesonet network (Bowling Green, KY).

2.2 | Experimental design

A randomized complete block design with four replications and a two-factor factorial treatment arrangement was utilized for this study. Field position was used as a blocking factor due to presence of very gentle slopes. Plots within blocks

TABLE 1 Forage species, cultivars, and seeding rates for each treatment

Treatment	Scientific name	Cultivar	Seeding rate ^a kg ha ⁻¹
Monoculture			
Sudangrass	<i>Sorghum bicolor</i> (L.) Moench ssp. <i>drummondii</i> (Nees ex Steud.) de Wet & Harlan	AS9302	56
Simple			
Sudangrass	See above		28
Pearl millet	<i>Pennisetum glaucum</i> (L.) R. Br.	Wonderleaf	5.6
Soybean	<i>Glycine max</i> (L.) Merr.	Large Lad	28
		Total	61.6
Complex			
Sudangrass	See above		15.7
Pearl millet	See above		4.5
Crabgrass	<i>Digitaria ciliaris</i> (Retz.) Koeler and <i>Digitaria sanguinalis</i> (L.) Scop.	Red River and Quick-N-Big	1.1
Corn	<i>Zea mays</i> L.	AgriGold 115 day	11.2
Soybean	See above		11.2
Cowpea	<i>Vigna unguiculate</i> (L.) Walp.	Red Ripper	11.2
Korean lespedeza	<i>Kummerowia stipulacea</i> (Maxim) Makino	VNS	4.5
Sunn hemp	<i>Crotalaria juncea</i> L.	VNS	2.2
Forage rape	<i>Brassica napus</i> L.	T-Raptor	1.1
Daikon radish	<i>Raphanus sativus</i> L.	SF Select	2.2
Sunflower	<i>Helianthus annuus</i> L.	Peredovik	2.2
		Total	67.2

^aPure live seed calculations not used because pure seed was above 98% and germination above 85% as per American Organization of Seed Certifying Agencies standards.

measured 2.7 by 6 m with 1.5-m alleys between blocks. New plot area was used each year.

Factors of interest were forage mixture complexity and N fertilizer rate. Forage mixture complexity consisted of three treatments: (a) summer annual grass monoculture (control); (b) simple mixture consisting of two summer annual grasses + one summer annual legume; and (c) complex mixture containing four summer annual grasses, four summer annual legumes, two brassicas, and one summer annual forb. Mixtures were inoculated with a peat-based multi-species inoculant (Link Cover Crop Inoculant, La Crosse Seed). Species, cultivars, and seeding rates used can be found in Table 1. Nitrogen fertilizer as ammonium nitrate was hand applied in split applications for each treatment and is depicted in Table 2. For the 56, 112, and 168 kg N ha⁻¹ treatments, N was applied in 56 kg N ha⁻¹ increments. All three treatments received an application at planting, while the 112 and 168 kg N ha⁻¹ treatments received another application after the first harvest. The 168 kg N ha⁻¹ received an additional application following the second harvest. Forty percent (90 kg N ha⁻¹) of the N fertilizer rate needed for the 224 kg N ha⁻¹ treatment was applied each at planting and after the first harvest, with the remaining

TABLE 2 Nitrogen application schedule and rates

Nitrogen treatment	N Applied		
	At planting	After first harvest	After second harvest
	kg N ha ⁻¹		
0 kg N ha ⁻¹	–	–	–
56 kg N ha ⁻¹	56	–	–
112 kg N ha ⁻¹	56	56	–
168 kg N ha ⁻¹	56	56	56
224 kg N ha ⁻¹	90	90	44

20% (44 kg N ha⁻¹) applied after the second harvest. Nitrogen fertilizer was split applied in this manner as it was similar to how a producer might apply N fertilizer at different rates, with lower N fertilizer rates applied in fewer applications as compared to higher rates.

2.3 | Plot management

In late May 2018 and early May 2019, plot area was sprayed with 2.3 kg glyphosate [N-(phosphonomethyl) glycine] ha⁻¹

TABLE 3 Soil test results and nutrient recommendations (applied as triple superphosphate and muriate of potash) for plots in Lexington, KY, and Princeton, KY, in 2018 and 2019

Environment	Soil test results			Amendments applied		
	Soil water pH	P	K	Lime	P ₂ O ₅	K ₂ O
		kg ha ⁻¹		Mg ha ⁻¹	kg ha ⁻¹	
2018 Lexington	7.1	141	417	0	0	0
2018 Princeton	7.2	66	207	0	34	123
2019 Lexington	5.4	353	136	4.75	0	179
2019 Princeton	7.2	11	195	0	123	146

twice, with approximately 2 wk between applications, to control existing perennial cool-season sod. Based on soil test results (Table 3), plot area was then fertilized with triple superphosphate (0–45–0) and muriate of potash (0–0–60) as needed to meet warm-season forage fertility requirements in accordance with the University of Kentucky Cooperative Extension Service 2018–2019 Lime and Nutrient Recommendations (Ritchey & McGrath, 2020).

Conventional seedbeds were prepared by rotovating followed by field cultivating in Lexington and by disking followed by field cultivating in Princeton until soil was fine and firm. Plots were planted approximately 1 mo following the last herbicide application using a small plot walk-behind cultipack-type seeder (Carter Manufacturing) on the following dates for each location: 27 June 2018 and 5 June 2019 at Lexington and 19 June 2018 and 11 June 2019 at Princeton.

Prior to harvest, plant height was measured with a leveling rod (SVR Series, Seco Industries). One measurement was taken at the end of each plot by estimating average height of all plants in plot. Height of tallest leaves or seed heads (if present) were recorded. Harvests were targeted to occur at a plant height of approximately 75–100 cm, however, some harvests were delayed resulting in greater heights. Harvest occurred on the following dates: 15 Aug. 2018, 20 Sept. 2018, 25 Oct. 2018, 11 July 2019, 7 Aug. 2019, and 20 Sept. 2019 at Lexington and 2 Aug. 2018, 7 Sept. 2018, 9 Oct. 2018, 19 July 2019, 19 Aug. 2019, and 3 Oct. 2019 at Princeton. A 1.5-m strip was clipped through the center of the plot using a Hege 212 small-plot forage harvester (Wintersteiger Inc.) leaving 20 cm of residual after the first and second harvests, and 10 cm of residual after the final harvest. Entire plot area was cleared after harvest.

Fresh material was weighed upon harvest, and two 250-g subsamples were collected from each plot, one for botanical separation and one for dry matter determination. Subsamples were weighed fresh and dried in a forced air oven for 5–7 d at 55 °C until a constant weight. Percentage dry matter was calculated as follows: (dry weight/fresh weight) × 100. Total yield was calculated using the following equation: kg dry mat-

ter ha⁻¹ = (kg fresh plot weight 9 m⁻²) × 10,000 m² ha⁻¹ × (% dry matter/100).

Following harvest, botanical samples were refrigerated until separations could occur. Samples were separated into each individual planted species with an additional category for weeds (anything not planted). Botanical components were then oven dried and each component's percentage of the sward on a dry matter basis was calculated using the following equation: (individual component mass/total component mass) × 100. Yield of each component was then determined by multiplying the component's proportion by the plot dry matter (DM) yield.

2.4 | Data analysis

Data were analyzed using SAS 9.4 software (SAS Institute). The general linear model procedure was used to generate ANOVA tables and means were separated using Fisher's protected least significant difference post-hoc test. Treatments, year, and location were considered fixed effects. Year × location interactions were observed, and data are presented by environment (year × location combination). No treatment interactions (N fertilizer rate × mixture) were observed, therefore main effects are presented. Regression models were determined using orthogonal polynomial contrasts and regression analyses were performed using the REG procedure on the appropriate contrast which was selected using the backward elimination method. A significance level of $\alpha = .05$ was used for all analyses.

3 | RESULTS AND DISCUSSION

Monthly temperature and precipitation averages for both Princeton and Lexington from 2018 and 2019 are compared with the most recent 30-yr climate normals (1981–2010; NOAA National Centers for Environmental Information, Ashville, NC) and are shown in Figure 1. As the growing season for warm-season annual forages in Kentucky is

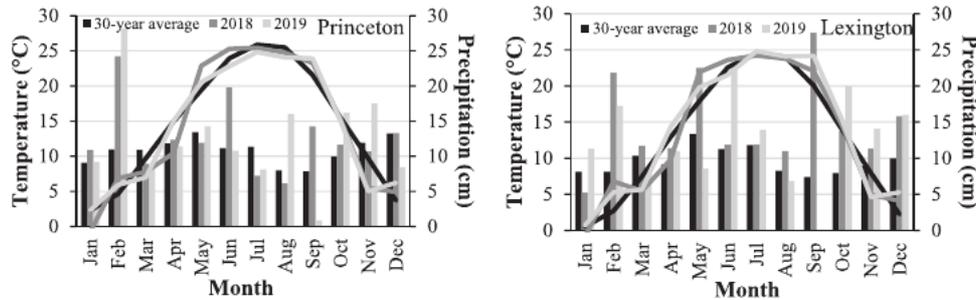


FIGURE 1 These climographs depict average monthly precipitation (bars) and temperature (lines) for 2018 and 2019 (Kentucky Mesonet, Bowling Green, KY), compared with the 30-yr climate normals (1989–2010; NOAA National Centers for Environmental Information, Ashville, NC) for both (left) Princeton, KY, and (right) Lexington, KY

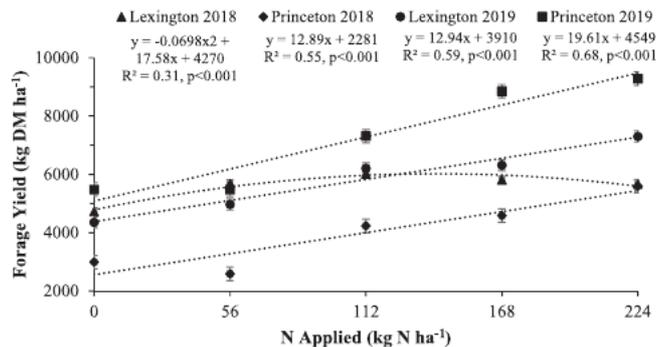


FIGURE 2 Impact of N fertilizer rate averaged over forage mixtures (no mixture \times N fertilizer rate interaction) on total forage dry matter for Lexington 2018, Princeton 2018, Lexington 2019, and Princeton 2019

typically May–October, the following weather information is summarized for that time period only. Lexington had nearly 40 cm greater rainfall in 2018 and 13 cm greater rainfall in 2019 than the 30-yr average of 60 cm. For both those years, temperatures in Lexington were approximately one degree warmer than the 30-yr average of 20.5 °C. Temperature and rainfall for Princeton in 2018 and 2019 were similar to the long-term average of 62 cm and 22.0 °C. Rainfall in both locations was above average in September 2018 and far below average in September 2019.

3.1 | Nitrogen fertilizer rate effect on biomass production

For all environments, N fertilizer rate significantly impacted annual forage DM production ($p < .001$). In all but Lexington 2018, annual forage DM production increased in a linear trend as N fertilizer rate increased. In Lexington 2018, annual forage DM production peaked at 112 kg N ha⁻¹ and then declined (Figure 2).

Nitrogen is often the most limiting factor in biomass production (Vitousek & Howarth, 1991). The positive relation-

ship between N fertilizer rate and yield was to be expected, and was similar to the results of Tofinga (1990), who concluded that N was the strongest determinant of yield and quality of grass–legume intercrops. Positive effects of N application on yield of grass–legume intercrops has also been shown in corn and ricebean [*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi]; Rerkasem & Rerkasem, 1988), sorghum and cowpea (Patel & Rajagopal, 2003), and corn and cowpea (Asangla & Gohain, 2016).

The Lexington 2018 site did not show the same trends, however. There may have been some soil, management, or environmental properties unique to the site that was not measured but could have contributed to the limited yield response to N. For example, residual soil N or mineralizable N may have played a role in the limited yield response to N fertilizer at Lexington in 2018. Alfalfa was terminated at the Lexington 2018 site 3 yr prior to the establishment of the current study, with subsequent cropping of graminoid species occurring. This management history was not expected to impact treatment response to N fertilizer rates as length of time since legume termination was deemed adequate in conjunction with the high yearly precipitation that results in nitrates leaching from the root zone. Alternatively, above-average rainfall amounts may have leached N from the system, although crude protein of forages in that environment was nearly double that of all other environments (16.6 vs. 8.6%; unpublished data, 2021) implying that N was not limited in the system.

3.2 | Diversity effect on biomass production

Mixture complexity only affected annual forage DM production in Lexington 2018 ($p < .001$), where the simple mixture had greatest annual forage DM production (6,000 kg DM ha⁻¹), followed by the complex mixture (5,670 kg DM ha⁻¹), and the monoculture (5,060 kg DM ha⁻¹). This response was most likely due to greater pearl millet [*Pennisetum americanum* (L.) Leeke] biomass accumulation in mixtures in this environment as compared to other environments (see

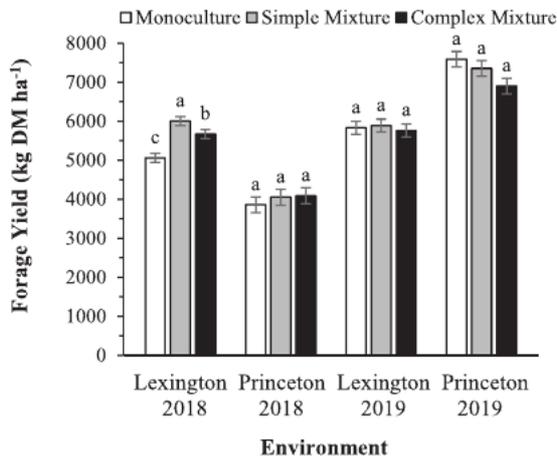


FIGURE 3 Impact of mixture complexity averaged over N fertilizer rates (no mixture \times N fertilizer rate interaction) on total annual forage dry matter production for each environment (location \times year interaction). Treatments within environment with the same letter are not different according to Fisher's Protected Least Significant Difference ($\alpha = .05$)

botanical composition results below). In all other environments, no differences in annual yield occurred between forage mixtures (Princeton 2018 = 4,000 kg DM ha⁻¹; Lexington 2019 = 5,830 kg DM ha⁻¹; and Princeton 2019 = 7,280 kg DM ha⁻¹; $p > .06$) (Figure 3).

Although generally not observed in the current study, increasing species diversity has often been linked to increased biomass production. This is often the case in native grasslands where competition for scarce resources is high and multi-species swards more efficiently utilize a variety of resources to fulfill differing niches (Minns et al., 2001). Lüscher et al. (2008) and Weigelt et al. (2009) even found this to be true in intensively managed grassland systems across Europe.

Conventional agricultural systems remain highly productive due to intensive management and competitive dominance of fast-growing species, even though biodiversity is generally low (DiTommaso & Aarssen, 1989). In the current study, intensive management (P and K fertility and harvest frequency) was employed to favor summer annual grasses. Early emergence and canopy closure of grasses promoted competitive dominance over the slower development of dicotyledonous species which lead to reduced diversity of swards in both mixtures (personal observation, 2019).

Numerous researchers have also shown positive diversity-productivity relationships in simple intercropping systems around the world. For example, Azraf-ul-Haq et al. (2007) observed 2.3 and 1.5 times greater yields of higher quality forage of sorghum intercropped with both cowpea and sesbania (*Sesbania sesban* L.), respectively, as compared to sole sorghum. Sharma et al. (2009) also reported a 22% increase in DM yield of sudangrass [*Sorghum bicolor* (L.) Moench \times

Sorghum sudanese (P.) Stapf]/cowpea mixtures over sudangrass monocultures.

Complex assemblages of warm-season annual forages are not as common to conventional agriculture and therefore less researched than simpler mixtures. In a study conducted in North Dakota, the authors observed greater biomass yield of a five species annual mixture of foxtail millet [*Setaria italica* (L.) P. Beauv.], forage sorghum blend, oat (*Avena sativa* L.), forage pea (*Pisum sativum* L.), and brassica hybrid Winfred (*Brassica napus* L.) as compared to monocultures of sorghum-sudangrass, sorghum, foxtail millet, and pearl millet (Mozea et al., 2020). This multispecies mixture may have overyielded in comparison to monocultures due to increasing functional diversity, as both cool- and warm-season annuals were present in the mixtures.

The mixtures in the current study may not have shown improved yield with increasing species diversity due to limited species and/or functional group evenness (see section on botanical composition results). Similar to the current results, in three- and four-way summer annual mixtures, Bybee-Finley et al. (2016) also showed no increase in yield of intercropping systems over the highest-producing monocrop in the northeastern United States. Similar to results of the current study, their plots were also dominated by summer annual grasses with a relatively low legume component.

In contrast to the above evidence, many studies have shown positive effects of intercropping, such as increased yield, land equivalency ratios, or economic returns (Andrews, 1972; Bybee-Finley et al., 2016; Fan et al., 2020; Takele et al., 2017). Researchers in Georgia even found that including crabgrass [*Digitaria sanguinalis* (L.) Scop.] in a pearl millet stand increased gains for finishing cattle by 0.12 kg d⁻¹ over pure stands of pearl millet (Harmon et al., 2019), while Sharma and colleagues (2009) reported 27% greater benefit to cost ratio of sudangrass/cowpea intercropped hay over sole sudangrass.

Weigelt et al. (2009) concluded that increasing biodiversity had an even stronger effect on biomass production of perennial grassland communities as compared to increasing management (N fertilization and mowing). In contrast, results from the current study found that in three out of four environments there was no improvement in biomass yield by increasing biodiversity, and positive responses to N application were observed. This indicates that in annual agricultural systems where mixtures are dominated by grass species, N is a stronger driver of biomass production as compared to increased biodiversity.

Weigelt et al. (2009) also observed greater yield responses to fertilizer when excluding legumes in mixtures, presumably due to the ability of leguminous species to share N acquired from biological N₂ fixation. As stated previously, this was not observed in the current experiment where no N fertilizer rate \times mixture complexity interaction occurred, resulting in each mixture responding to N fertilizer rate similarly, regardless

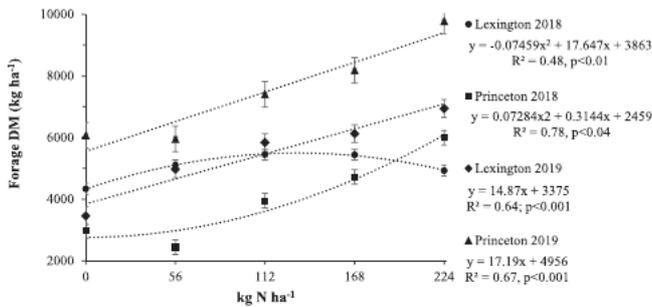


FIGURE 4 Sudangrass responses to N fertilizer rate in monoculture plantings at Lexington 2018, Princeton 2018, Lexington 2019, and Princeton 2019. Weed species did not respond to N fertilizer rate ($p > .08$) but contributed less than 500 kg dry matter ha^{-1}

of legume inclusion. In order to understand why three out of four environments in this study showed no yield advantage of intercropping, individual species contributions to sward DM were documented.

3.3 | Nitrogen fertilizer rate effect on botanical composition

Species responses in each mixture to N rate are presented for each environment in Figures 4–6 due to significant

N fertilizer rate x environment interactions. Only species with significant contributions to sward biomass are presented (>200 kg DM ha^{-1}).

3.3.1 | Monoculture

In Lexington 2018, sudangrass DM yield showed a significant, but limited quadratic response to increasing N fertilizer rate, with a maximum yield of 5,500 kg DM ha^{-1} occurring around 135 kg N ha^{-1} ($p < .01$, $R^2 = .48$; $y = -0.0746x^2 + 17.65x + 3,863$; Figure 4a). Weeds contributed 1% of sward DM. In Princeton 2018, sudangrass DM yield showed a slight quadratic response as N fertilizer rate increased, from 2,965 to 5,991 kg DM ha^{-1} ($p < .04$, $R^2 = .41$, $y = -0.0746 \times 2 + 17.65x + 3,863$; Figure 4b). Weed biomass accounted for only 1% of sward DM. In Lexington 2019, sudangrass DM linearly increased as N fertilizer rate increased, with a maximum yield of 6,640 kg DM ha^{-1} ($p < .001$; $R^2 = .64$; $y = 14.87x + 3,375$; Figure 4c). While weed DM averaged 306 kg DM ha^{-1} , it only contributed 5% of sward DM. In Princeton 2019, sudangrass DM linearly increased as N fertilizer rate increased, with a maximum yield of 9,790 kg DM ha^{-1} ($p < .001$; $R^2 = .67$; $y = 17.19x + 4,956$; Figure 4d). Weed proportion was 2% of sward DM.

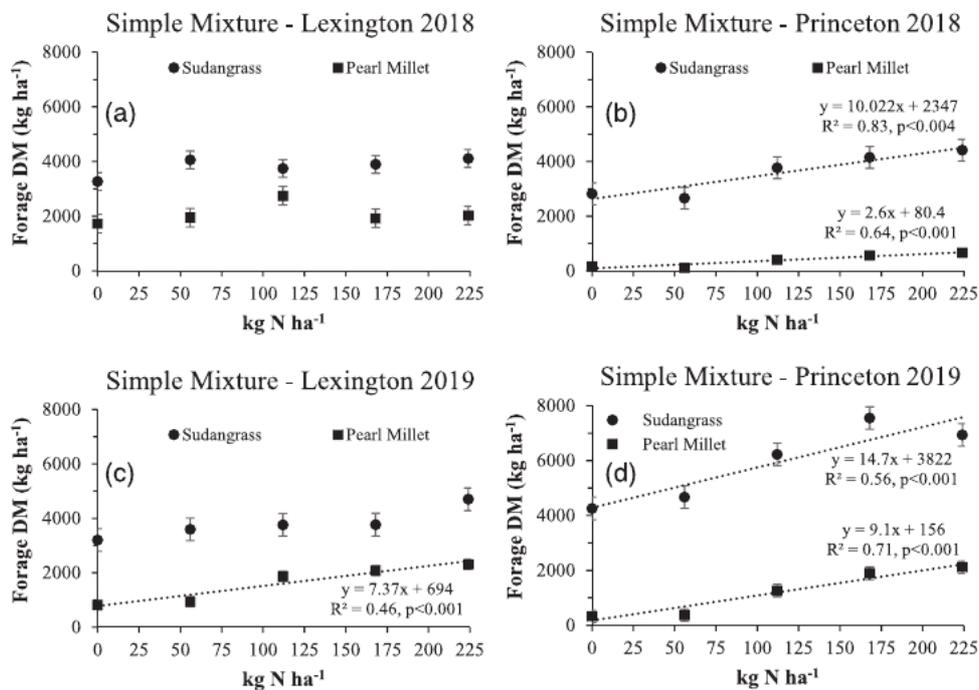


FIGURE 5 Responses of individual species in simple, three species forage mixtures to N fertilizer rates at (a) Lexington 2018, (b) Princeton 2018, (c) Lexington 2019, and (d) Princeton 2019. Soybean are not presented if they contributed <200 kg dry matter (DM) ha^{-1} to sward biomass. Weeds are not presented as they did not respond to N fertilizer rate. Species with no regression line did not respond to N fertilizer rate ($p > .05$). Lexington 2019 pearl millet error bars are not shown due to small size (185 kg DM ha^{-1})

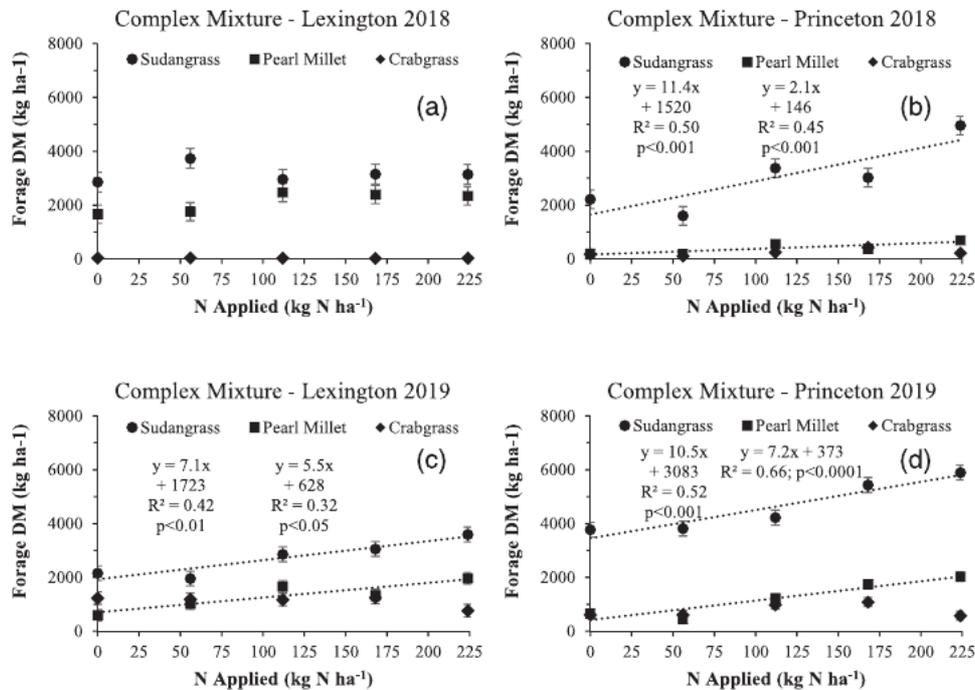


FIGURE 6 Responses of individual species in a complex, 12-species mixture to N fertilizer rate at (a) Lexington 2018, (b) Princeton 2018, (c) Lexington 2019, and (d) Princeton 2019. Weeds, soybean, corn, sunflower, sunn hemp, forage rape, daikon radish, and Korean lespedeza contributed <200 kg dry matter (DM) ha^{-1} to sward DM and are not presented. Species with no regression line did not respond to N fertilizer rate ($p > .05$). Error bars not shown due to small size are as follows: 10, 69, 69, 163, and 162 kg DM ha^{-1} for Lexington 2018 crabgrass, Princeton 2018 pearl millet and crabgrass, and Princeton 2019 pearl millet and crabgrass, respectively

3.3.2 | Simple mixture

In Lexington 2018, no individual species responded to increasing N fertilizer rates ($p > .20$). Sudangrass and pearl millet averaged 3,410 and 1,850 kg DM ha^{-1} (Figure 5a). Botanical composition was as follows: sudangrass = 64%, pearl millet = 35%, and soybean and weeds = 1% each.

In Princeton 2018, sudangrass ($p < .004$; $R^2 = .83$; $y = 8.3x + 2,346$) and pearl millet ($p < .001$; $R^2 = .64$; $y = 2.6x + 80.4$) DM increased with increasing N fertilization rate, with maximum DM yields of 4,412 and 667 kg DM ha^{-1} (Figure 5b). Botanical composition was as follows: sudangrass = 87%, pearl millet = 9%, soybean = 1%, and weeds = 2%.

In Lexington 2019, pearl millet was the only species to respond to N fertilizer rate, with a maximum yield of 1,600 kg DM ha^{-1} ($p < .001$; $R^2 = .46$; $y = 7.37x + 694$). Sudangrass and weeds averaged 3,810 and 361 kg DM ha^{-1} , respectively (Figure 5c). Botanical composition was as follows: sudangrass = 65%, pearl millet = 27%, soybean = 2%, and weeds = 6%.

In Princeton 2019, sudangrass ($p < .001$; $R^2 = .56$; $y = 14.7x + 3,822$) and pearl millet ($p < .001$; $R^2 = .71$; $y = 9.1x + 156$) yields increased with increasing N fertilizer rate, with maximum yields of 6,940 and 2,120 kg DM ha^{-1} , respectively (Figure 5d). Botanical composition was as fol-

lows: sudangrass = 75%, pearl millet = 23%, and soybean and weeds = 1% each.

3.3.3 | Complex mixture

In Lexington 2018, no individual species ($p > .06$) responded to N fertilizer rate in Lexington 2018, but sudangrass and pearl millet averaged 3,170 and 2,120 kg DM ha^{-1} , respectively (Figure 6a). Botanical composition was as follows: sudangrass = 56%, pearl millet = 37%, corn = 2%, soybean, cowpea, sunflower (*Helianthus annuus* L.), and daikon radish (*Raphanus sativus* L.) = 1% each. Weeds, crabgrass, sunn hemp (*Crotalaria juncea* L.), forage rape (*Brassica napus* L.), and Korean lespedeza [*Kummerowia stipulacea* (Maxim) Makino] were not present.

In Princeton 2018, sudangrass ($p < .001$; $R^2 = .50$; $y = 11.4x + 1,520$) and pearl millet ($p < .001$; $R^2 = .45$; $y = 2.1x + 146$) increased as N fertilizer rate increased, ranging from 2,210 to 4,960 and 190 to 690 kg DM ha^{-1} , respectively, from 0 to 224 kg N ha^{-1} (Figure 6b). Botanical composition was as follows: sudangrass = 76%, pearl millet = 11%, crabgrass = 6%, and soybean, weeds, corn, cowpea, sunflower, sunn hemp, and Korean lespedeza = 1% each. Daikon radish and forage rape were not present.

In Lexington 2019, sudangrass ($p < .01$; $R^2 = .42$; $y = 7.1x + 1,723$) and pearl millet ($p < .05$; $R^2 = .32$; $y = 5.5x + 628$) increased with increasing N fertilizer rate (2,160–3,590 and 590–1,960 kg DM ha⁻¹, respectively). Crabgrass did not respond to N fertilizer rate ($p > .2$) but averaged 1,120 kg DM ha⁻¹. Crabgrass contributed >20% of the sward DM at rates of 0–168 kg N ha⁻¹, and even at 224 kg N ha⁻¹ contributed 11% of sward DM (Figure 6c). Botanical composition was as follows: sudangrass = 47%, pearl millet = 23%, crabgrass = 19%, corn = 4%, weeds = 2%, and soybean, cowpea, sunflower, and Korean lespedeza = 1% each. Sunn hemp, daikon radish, and forage rape were not present.

In Princeton 2019, sudangrass ($p < .001$; $R^2 = .52$; $y = 10.5x + 3,083$) and pearl millet ($p < .0001$; $R^2 = .66$; $y = 7.2x + 373$) DM yield increased with increasing N fertilizer rate, with maximum yields of 5,900 and 2,030 kg DM ha⁻¹, respectively. Crabgrass DM was not affected by N fertilizer rate ($p > .1$) but contributed 770 kg DM ha⁻¹ on average. Crabgrass contributed >11% sward DM in all N fertilizer rates up to 168 kg N ha⁻¹. At 224 kg N ha⁻¹, crabgrass contributed 7% of sward DM (Figure 6d). Botanical composition was as follows: sudangrass = 67%, pearl millet = 18%, crabgrass = 11%, and soybean, weeds, corn, cowpea, and sunn hemp = 1% each. Sunflower, daikon radish, forage rape, and Korean lespedeza were not present.

3.3.4 | Nitrogen fertilizer rate effect on grasses

For most environments and mixtures, sudangrass and pearl millet biomass responded positively to N fertilization and were most likely the driver of sward responses to N application, as most other species contributed minimally to sward biomass. Pearl millet performed moderately well in both mixtures in Lexington, even though seeds planted per hectare were 62 and 88% of that of sudangrass in simple and complex mixtures, respectively. Weed components were consistently low and did not respond to N fertilizer application. This implies that planted driver species were more competitive than weeds in acquiring N and other resources in these environments.

Sudangrass seeding rates were somewhat higher than recommended for grazing but were within recommendations for hay, as much higher seeding rates have been recommended in the southwestern United States to produce finer-stemmed hay (Knowles & Ottman, 2015). These high-planting populations most likely contributed to mixtures being overwhelmingly dominated by sudangrass, leading to reduced competitiveness of other component species. Osiru and Willey (1972) conducted an experiment using sorghum and common bean

(*Phaseolus vulgaris* L.) mixtures in differing seeding proportions. Their results indicated that yields of components in mixtures were affected by seeding proportion of individual species. Craufurd (2000) also observed cowpea yield declining with increasing grass density, even though overall intercrop yield was still increased as compared to monocultures.

These findings, in accordance with results of the current experiment, reinforce the need for balanced seeding rates to achieve target goals (i.e., diversity, yield, etc.). Botanical separation results in the current study may have differed if seeding rates of secondary species were increased or if seeding rates of the dominant, highest-yielding species were decreased. However, it is unclear as to what degree, as tillering response can increase with decreased seeding rate, leading to yield compensation (Sowiński & Szydełko, 2011).

Although crabgrass comprised a substantial proportion of sward DM in only two environments (7 and 20%), it may be useful to include in annual warm-season forage mixtures. In the current study, the low crabgrass composition was likely a result of harvest management in the current study. In order to favor higher-yielding grass species, cutting height was set to 18–20 cm for the first two harvests. During the first harvest, crabgrass was minimally present, but proportions increased at each successive harvest (data not shown), likely due to increased light availability following defoliation of taller species. Crabgrass typically produces greater biomass under shorter defoliation heights, as residual plant height following mowing or grazing is recommended at 7–15 cm (Blount et al., 2003). A reduced cutting height may also have reduced competitive advantage of taller-growing summer annuals, thereby leaving more resources available for crabgrass growth.

Although management of this experiment did not include grazing, animals have been observed lowering their heads to graze smaller-statured species such as crabgrass and brassicas amid a mixed pasture including tall-growing species such as sorghum–sudangrass and pearl millet (personal observation, 2017). Crabgrass also regularly “fills in” thin spots in perennial pastures and can be a good way to increase groundcover and perhaps overall pasture yield. More research is needed to evaluate diet selection, animal performance, and regrowth potential of grazed annual warm-season forage mixtures.

3.3.5 | Nitrogen fertilizer rate effect on legumes

Nitrogen fertilizer application has been shown to reduce soybean yield of intercrops due to the increased competitiveness of associated summer annual grasses (Layek et al., 2015). Unfortunately, this was not investigated in the current experiment due to low legume proportions (1 and 4% of sward DM in simple and complex mixtures, averaged across

TABLE 4 Pre-harvest height of monoculture, simple mixture, and complex mixture treatments. Means within rows sharing the same letter are not different ($p < .05$)

Environment	Harvest	Pre-harvest height			SE
		Monoculture	Simple mixture	Complex mixture	
Lexington 2018	1	1.03 a	0.99 a	0.99 a	0.02
	2	1.13 c	1.33 b	1.39 a	0.02
	3	0.25 b	0.29 a	0.30 a	0.01
Princeton 2018	1	0.91 a	0.91 a	0.90 a	0.01
	2	0.77 b	0.85 a	0.87 a	0.02
	3	0.52 a	0.53 a	0.51 a	0.01
Lexington 2019	1	0.88 a	0.85 a	0.84 a	0.02
	2	0.65 b	0.78 a	0.82 a	0.02
	3	0.83 ab	0.87 a	0.77 b	0.03
Princeton 2019	1	0.75 a	0.78 a	0.73 a	0.02
	2	0.91 b	1.00 a	0.97 a	0.02
	3	1.20 a	1.24 a	1.24 a	0.03

environments). It was noted that N deficiency in low N fertilizer rate treatments was more pronounced prior to second and third harvests, as compared to the first cutting (personal observation, 2019). Perhaps there was adequate residual soil N to support vigorous grass growth early in the growing season, even in 0 kg N ha^{-1} treatments, leading the grasses to outcompete the legumes.

Some researchers have speculated that soybean is not compatible when grown in association with warm-season grasses due to shading from the taller-statured species, resulting in loss of photosynthetic activity, nodulation, and N_2 fixation (Brainard et al., 2011). Reddy et al. (1990), Gilbert et al. (2003), Wahua and Miller (1978) also observed increases in cowpea yield when planted with dwarf rather than tall pearl millet, furthering support of claims that legumes are at a disadvantage when grown with taller grasses. Alternatively, Layek et al. (2012) showed soybean to be compatible with sorghum and corn, but not pearl millet, due to its tillering nature. However, tillering sudangrass and pearl millet were specifically selected for the current study due to their regrowth potential under multi-cut management.

The multi-cut system used in this experiment also favored grasses over dicotyledonous species, reinforcing the idea that species selection should match management strategy. Results of the current study would likely have been affected by using dwarf grass varieties. Seeding legumes earlier than grasses has been shown to be a viable way of allowing the legume time to emerge before being outcompeted by grasses (Iqbal et al., 2017), as legume growth rates are typically less than those of grasses (Bybee-Finley et al., 2016). Unfortunately, this strategy may not be feasible or practical in most agricultural operations.

3.4 | Pre-harvest height

No consistent differences were observed for height of mixtures prior to harvest (Table 4). In 6 out of 12 harvests, the complex mixture was taller than the monoculture ($p < .05$). In 5 out of the 12 harvests, the simple mixture height was also greater than the monoculture ($p < .05$). The remainder of the six harvests showed no difference in mixture height ($p > .24$).

Plot height was measured relative to the tallest growing species in the mixture. Both simple and complex mixtures contained pearl millet, which seems to be the driver of the height responses observed. Visual observations also indicated that most legumes were considerably shorter than sudangrass and pearl millet, with the exception of sunn hemp, which was the tallest growing legume in the complex mixture.

3.5 | Implications

Very few published studies have evaluated diverse mixtures of summer annual forages. However, some producers have had success with maintaining diversity in these systems (personal observation, 2019). This gap between scientific understanding of species compatibility and practical application leaves an opportunity to evaluate the agronomic, economic, and environmental benefits of diverse summer annual forage systems. Understanding species compatibility will ultimately lead to improved economics of these systems.

A prominent theory as to why low diversity swards have equal or greater biomass production as compared to higher diversity swards has been suggested by Picasso et al. (2008), Huston et al. (2000), and Sanderson et al. (2004). These

authors determined that the positive relationship between species richness and biomass is mainly a reflection of a strong influence of one or two well-adapted species in a community. The authors also stated that polyculture plots including high-producing forage species did not increase biomass when species diversity was increased, and that plots containing a dominant “driver” species yielded similarly to the same species grown in a monoculture.

These trends were also exhibited in the current study where in three out of four environments, mixtures did not outyield monocultures and were dominated by high-yielding species. The mixtures in the current study were dominated by species and cultivars that have been shown to yield well on the experimental sites. However, seeding rates of high-producing species were reduced in the complex mixture in order to “make room” for additional species. Seeding rates of these aggressive species could have been further reduced to allow less competitive species an opportunity to contribute more to sward biomass, although total biomass production may have been lower if seeding rates were significantly reduced.

The additional species in the complex mixture were selected to increase functional diversity, and unfortunately some species selected were not as competitive or well adapted to experimental sites and did not contribute significantly to sward biomass. As agriculture production relies on economic efficiency, planting less competitive species in a mixed sward would not be advised, especially if management strategies will not favor them. This could lead to an increased seed cost without a substantial production benefit.

In a multi-location trial, Barker et al. (2003) also found that different simple mixtures or monocultures obtained the highest yields at different locations. The authors recommend planting simple mixtures of high-yielding species as opposed to monocultures as it may be hard to determine which species will yield best on specific sites or within-site microclimates. Due to the competitive dominance of fast-establishing summer annual grasses, Bybee-Finley et al. (2016) also recommend matching species with similar plant heights and growth rates in intercropping systems to limit competition for sunlight. Additionally, the authors of the current study recommend reducing seeding rates of tall-growing grasses or utilizing dwarf cultivars in mixtures if species diversity is to be targeted. This would allow for more resources, particularly sunlight, to be partitioned to slower, or lower-growing species such as crabgrass and legumes that otherwise may not establish or compete well. Including species that are not well-suited to a management system could lead to an increased seed cost without a substantial production or diversity benefit; therefore, grass-only plantings may be more suitable under hay management regimes, as regrowth potential of annual legumes is generally less than that of grasses.

It was hypothesized that inclusion of legumes in this study would affect the mixture responses to N fertilization, and with

a strong enough legume component less N fertilizer would be required to produce similar yields as monoculture sudan-grass. This was not the case in the current study, as results showed similar yield trends for each mixture with increasing N fertilizer rate (no mixture x N fertilizer rate interaction). Legume content of these swards was admittedly low for the mixtures (<5%), which most likely contributed to these results. Due to low legume content from poor establishment and competition from taller grass species, N recommendations for legume-containing summer annual forage mixtures cannot be made based on the current study. However, other research has shown that grass–legume intercrops often still respond positively up to the 100% recommended N fertilizer rate for the grass species (Layek et al., 2015; Takele et al., 2017).

The current data and cited literature indicate that planting high-yielding, morphologically, and developmentally compatible species in simple mixtures and fertilizing according to the grass recommendations may be advantageous in regards to economic efficiency of summer annual forage systems. However, if forage diversity is the primary goal, reducing seeding rates or using dwarf cultivars of dominant species is imperative to allow less competitive species an opportunity to establish and make significant contributions to the overall biomass production. Additionally, Tracy and Faulkner (2006) stated that “compared with pasture species richness, grazing management and climatic conditions more strongly influence grazing system productivity.” In accordance with this statement, the yields in the current study were affected by environment, and no or limited yield benefits from increasing biodiversity were observed. Producers are encouraged to invest in grazing management infrastructure (fencing and water resources) for additional opportunities to increase biomass production rather than planting complex summer annual forage mixtures.

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AUTHOR CONTRIBUTIONS

Kelly M. Mercier: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Validation; Visualization; Writing-original draft; Writing-review & editing. Christopher D. Teutsch: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. S.

Ray Smith: Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Writing-review & editing. Edwin L. Ritchey: Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Writing-review & editing. Kenneth H. Burdine: Conceptualization; Investigation; Methodology; Project administration; Supervision; Writing-review & editing. Eric S. Vanzant: Conceptualization; Investigation; Methodology; Project administration; Supervision.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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