

BIOMASS YIELD AND NUTRITIVE VALUE OF ANNUAL FORAGE MIXES COMPARED
WITH MONOCROPS

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State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Forage mixes could serve as a cover crop mix to protect the soil during the winter, for prevented planting areas or as a high nutritious feed for grazing livestock. The objective of this study was to determine the nutritive value and productivity of selected annual forage mixes compared with forage sorghum monocrops. The nutritive value of annual forage mixes and monocrops varied across environments and between treatments. Monocultures produced more biomass than annual forage mixes. The three most productive mixes in comparison to the others were hybrid brassica/oat/forage pea/forage sorghum x sudangrass/sweet sorghum blend/foxtail millet, turnip/forage sorghum x sudangrass/sweet sorghum blend/forage pea/hybrid brassicas/oat/faba bean/forage pea, and forage sorghum x sudangrass/radish mix. The latter being the most cost-effective mix. Forage sorghum dominated annual forage mixes at a planting rate of 2.2 kg/ha. These results emphasize how forage annual mixes can provide additional forage for livestock.

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DEDICATION

To Family, for prayers, understanding and patience, especially my wife, Alicia for always backing me up. Finally, to every single child out there with the hope of having an education, you are not alone, may help locate you.

Life is a path, walk through it, trust the process and enjoy every moment of it.

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1. INTRODUCTION

Annual forages for fodder production are becoming more popular due to an increase in demand for pasture-raised cattle (Hoffman et al., 2015). Unlike perennial forages that have the tendency to become mature with low quality during late summer, annual forages extend the grazing period into fall, which allows beef producers to reduce feed costs and preserve hay for winter-feeding (Ketterings et al., 2015; McCartney and Baron, 2013).

Annual forages include grasses, legumes, brassicas, and other forbs. Feeding animals with a single annual crop has its own set of constraints (Farney et al., 2018). In Minnesota, for instance, solely grazed forage sorghum [*Sorghum bicolor* (L.) Moench] can cause prussic acid poisoning if grazed or fed after a cutting or light frost, despite being a high-yielding forage. Annual legumes such as pea (*Pisum sativum* L.) are a protein source for cattle but can cause bloat if it is the only source of feed (Lalman, 2014). Another example is that ruminants fed only forage brassicas can get diarrhea and develop cerebrocortical necrosis if they are fed sulfur-rich brassica diets (Lenz et al., 2017; McKenzie et al., 2009). Brassicas can also induce bloating because of their easily digested carbohydrates, proteins, and low fiber content (Lenz et al., 2017). As a result, Barry (2013) advised that brassicas be fed to ruminants in mixes with other high fiber forages.

Grasses, legumes, and brassicas are commonly planted in mixes to balance nutritive value, fiber and productivity (Bainard et al., 2020). These annual forage mixes have the capacity to enhance forage nutritive value when compared with monocrops by providing a well-balanced diet that meets animal requirements of protein, digestible fiber, and energy. Three annual forage mixes were evaluated and compared with a monocrop of oat (*Avena sativa* L.) (Bainard et al., 2020). The three-species mixes had substantially higher nutritive value than oats alone. The

mixtures were a three-species mix [oat, forage pea, and forage radish (*Raphanus sativus* L.)], a six-species mix [oat, annual ryegrass (*Lolium multiflorum* L.), forage pea, hairy vetch (*Vicia villosa* Roth), forage radish, and a hybrid brassica (*Brassica rapa* L. x *B. oleraceae* L.)], and a nine-species [six-species mix + forage sorghum, crimson clover (*Trifolium incarnatum* L.) and a hybrid brassica (*Brassica rapa* L. x *B. napus* L.)].

Sanderson et al. (2018) compared the biomass yield of 15 combinations of four annual forage species including proso millet (*Panicum milleaceum* L.), triticale (*Triticosecale* x Wittm.), red clover (*Trifolium pratense* L.), and radish with each species as a monoculture for three years in a study conducted in North Dakota. Annual mixes and monocultures had a mean average biomass yield of 2.4 Mg ha⁻¹ and 1.7 Mg ha⁻¹, respectively, although planting dates and seeding rates had a strong effect on productivity and establishment.

Annual forage mixes can provide economic and environmental benefits when properly managed, since they have the ability to provide high forage yield and nutritive value (Dillard et al., 2018). Forage-finished cattle have shown to be profitable when given the opportunity to graze a variety of forages (Harmon et al., 2019).

Previous research have compared the production and nutritive value of forage mixes with monocrops, although the bulk of these studies focused on incorporating annual forage mixes into existing established cash crops stands for grazing, hay, or crop rotation systems (Hansen et al., 2015; Villalobos and Brummer 2017; Wortman et al., 2012; Sedivec et al., 2011; Sedivec et al., 2020).

In this study, we hypothesized that annual mixes of brassicas, legumes, and grasses will have greater biomass yield and nutritive value compared with forage pearl millet (*Cenchrus americanus* (L.) Morrone) monocrop and forage sorghum x sudangrass/sweet sorghum

monocrops. In addition, we hypothesized that the seed cost per dry matter biomass produced from the annual forage mixes will be greater than that of the sorghum x sudangrass/sweet sorghum and pearl millet.

The subject of this study was to determine the differences in biomass yield, nutritive value, and cost per dry matter yield between forage sorghum/sudangrass/pearl millet monocrops and annual forage mixes.

1.1. Objectives

- To evaluate which annual forage mixture provides the highest biomass production during the first and second harvest when compared with monocrops.
- Compare the nutritive value of annual forage mixes to monocrops to see if they meet the nutritional demands of grazing animals.
- To assess the difference between the cost per megagram of dry matter biomass yield produced by annual forage mixes and sorghum monocrops.

2. LITERATURE REVIEW

2.1. Benefit of Forages for Animal Performance

Plants that are utilized as cattle feed whether wild or cultivated, are known as forages. Forages for cattle can be grasses and broadleaf vascular plants such as legumes, plants in the *Brassicaceae* family (henceforth brassicas), shrubs, and even some trees, depending on the region. Forages also account for more than 80% of total feed utilized in beef cattle production in North America (Beauchemin et al., 2010).

For grazing animals, forage is an essential source of protein and energy, and the forage species or mixes of them have an impact on their performance. The performance of grazing animals is evaluated mainly by animal weight gain (Newman et al., 2013). Previous studies have illustrated the relationship between forage species and animal performance. Maughan et al. (2014) reported the influence of forage species in a grass-legume mixture on the meat quality of cattle, where cows (*Bos taurus* L.) that grazed on tall fescue (*Schenodorus arundinacea* Schreb.)/sainfoin (*Onobrychis viciifolia* S.) produced darker red meat to cows that grazed on tall fescue/alfalfa mixture (*Medicago sativa* L.).

Turnip (*Brassica rapa* L.) fed lambs (*Ovis aries* L.) exhibited considerably greater body weight (BW) and average daily gain (ADG) than oat fed lambs, indicating that forage species have an impact on ruminant body weight (Campbell et al., 2021). In addition, brown mid-rib (BMR) sorghum x sudangrass (*Sorghum bicolor* L. x *S. sudanense* Desv.) and pearl millet had an average daily gain (ADG) of 0.99 kg d⁻¹ and 0.85 kg d⁻¹ in finishing cattle, respectively (Harmon et al., 2019).

2.2. Annual Forage

Annual forages are plants that mature in a single growing season and are seeded on land to be grazed, hayed, or ensiled. Most of these annual forages are either warm-season or cool-season crops (Drewnoski and Redfearn, 2017). The planting date is the most significant distinction between these annual forage types. Warm-season plants are seeded in late spring, whereas cool-season crops are planted early in the spring or in the fall (Drewnoski and Redfearn, 2015). Farmers create full-season annual forage mixes combining cool-season and warm-season forages or, in certain cases, double-cropping of annual forages in order to have available forage year-round (Volesky and Drewnoski, 2016).

An annual forage mix typically has more than two annual forage species. It combines multiple species with unique ecological functions, such as nutrient scavenging, biological nitrogen fixation, different growth habit, morphology, and chemical composition that complement each other, resulting in improved forage nutritive value and vegetative soil cover (Díaz and Cabido, 2001). A nutritive annual grazing mix often contains at least one of each grass, legume, and brassica (Sedivec et al., 2020). Grasses in mixes are high in dry matter and fiber and have greater fiber digestibility than legumes. Forage legumes provide more protein than grasses, and brassicas have very high digestible matter and retain their nutritive value until late fall, whereas monocrops can only provide part of the nutritive requirements of cattle depending on the sown species (Islam et al., 2013; McCartney and Baron, 2013; Titlow et al., 2014).

2.3. Annual Forage Mixes Biomass Yield Compared with Monocrops

Greater diversity of species leads to more efficient resource usage; as a result, some monocultures may produce less biomass than diversified forage mixes; however this depends on the environment they are grown (Mirsky et al., 2013; Smith et al., 2014). Establishing and

managing annual forage mixes can be difficult, especially if species have different seed size, growth rate, herbicide tolerance, and harvest requirement, as well as the possibility of intraspecific competition (Wortman et al., 2013).

Wortman et al. (2012) conducted a study on annual forage mixes productivity and stability in the western Corn Belt in Nebraska and observed that mixes containing legumes (hairy vetch, field pea, crimson clover, chickling vetch [*Lathyrus sativus* L.]), mustards (*Sinapis alba* L., and *Brassica juncea* L.), and other brassicas (forage rape and radish) had about twice as much forage yield than the yield of hairy vetch, field pea, and crimson clover monocrops.

When comparing yield of annual ryegrass monocrop with annual forage mixes comprising of annual ryegrass, red clover (*Trifolium pratense* L.), and balansa clover (*Trifolium michelianum* L.). Ryan-Salter and Black (2012) in a study conducted in New Zealand, discovered that mixed annual forages had greater yield than solely-planted forage species. The yield of the annual ryegrass/balansa clover/red clover mix was 13.2 Mg dry matter (DM) ha⁻¹, which was 35% greater than the yield of the annual ryegrass monocrop Ryan-Salter and Black, 2012).

The majority of research on annual forage mixes focuses on their influence on late-season grazing, integration into existing perennials or cash crops, and crop rotation systems (Titlow et al., 2014; Wortman et al., 2012; Villalobos and Brummer 2017; Liebig et al., 2015). Hence, factors like planting date and location should be considered when comparing the forage yield of different studies.

For example, the biomass yield of a late-summer planted mixture comprising turnip, proso millet, triticale, soybean [*Glycine max* (L.) Merr.], pea, and canola (*Brassica napus* L.)

was not different from the biomass yield of each individual species present in the mix as monocrops in Mandan, ND (Liebig et al., 2015).

Annual forage mixes act as a soil cover, preventing nutrients leaching and runoff, protecting water quality, and storing nitrogen in the biomass without reducing the yield of the following cash crop (Noland et al., 2018). A mixture of winter rye (*Secale cereale* L.), radish, winter pea (*Pisum sativum* ssp. *arvense* L.), and camelina [*Camelina sativa* (L.) Crantz] produced a biomass yield of 2.3 Mg DM ha⁻¹ when interseeded into standing soybean, while the biomass yield of the soybean 2.95 Mg DM ha⁻¹ which was not different from soybean that had no interseeded cover crops (Peterson et al., 2019).

2.4. Forage Nutritive Value Between Mixes and Monocrops

Sanderson et al. (2018) reported that annual forage mixes containing pearl millet, triticale, radish, and red clover had significant greater crude protein content than monocrops of pearl millet and triticale. This is like the results in a study in which several mixes were compared with monocrops (Omokanye et al., 2019). The mixes were a 9-species mix (hairy vetch, annual ryegrass, BMR sorghum, crimson clover, forage rape (*Brassica napus* L.), radish, forage pea, and oat), a 7-species mix [hairy vetch, sorghum x sudangrass, triticale, berseem clover (*Trifolium alexandrinum*), forage brassica, oat, and forage pea], and a 5-species mix (annual ryegrass, hairy vetch, turnip, forage brassica, and oat), which had greater impact on nutritive value than monocrops of oat, barley, triticale, and wheat (*Triticum aestivum* L.) (Omokanye et al., 2019). In both experiments, mixes including grasses, legumes, and brassicas outperformed monocrops in forage nutritive value.

2.5. Forage Sorghum, Pearl Millet, and Sorghum x Sudangrass/Sweet Sorghum, as Monocrops

Warm-season grasses such as forage pearl millet, sorghum x sudangrass, and forage sorghum are used extensively for grazing which is attributed to the high biomass yield accumulated by these crops over a brief period (Harmon et al., 2019). These crops can also be used as hay, silage, and native-pasture supplements (McCuistion et al., 2011). In addition, they are drought tolerant and require low inputs to maximize productivity (Machicek et al., 2019).

Forage mixes containing forage sorghum have greater biomass yield than mixes without it, which makes it a high-value forage crop in agronomic systems (Hassan et al., 2015). Forage sorghum has been reported as the feedstock crop with the highest biomass yield in North Dakota (Berti et al., 2013). In a double-relay cropping experiment, solely planted forage sorghum yielded the highest biomass of 26.2 and 19.7 Mg ha⁻¹ in Minnesota and North Dakota, respectively, however these yields were not different from maize (*Zea mays* L.) at 23.9 and 20.3 DM Mg ha⁻¹, respectively (Berti et al., 2015).

When examining the biomass yield of annual forages preceded by cover crops, Samarappuli et al. (2014) found the overall average yield of forage sorghum, averaged across four environments in North Dakota, was 18 Mg DM ha⁻¹. After 80 days of planting. Marsalis (2010) reported the yield of forage sorghum as 21 Mg DM ha⁻¹ in a trial that involved the comparison between the yield of forage sorghum and maize at different nitrogen rates.

Although a high yielding forage crop, forage sorghum generates hydrocyanic acid (prussic acid) as a response of stress or physical damage (frost and grazing), which is poisonous to ruminants but volatilizes out after 14 days (Lauriault et al., 2021). Pearl millet, unlike forage sorghum and sudangrass, does not produce hydrocyanic acid nor tannins, which makes it more

desirable for grazing (Assis et al., 2018). Research on forage resources for biofuel industry in North Dakota showed pearl millet had a total biomass yield of 22.6 Mg ha⁻¹ across two locations (Berti et al., 2013).

In a three-year study, average biomass output of pearl millet and sorghum x sudangrass for forage-finishing cattle systems was the same with both crops yielding 13.2 Mg DM ha⁻¹ in Athens, GA (Harmon et al., 2019). Lauriault et al. (2021) reported pearl millet and sorghum x sudangrass forage yield as 9.2 and 11.6 Mg ha⁻¹, respectively, in Tucumcari, NM.

2.6. Importance of Annual Forages and Forage Mixes to the Environment

Annual forage mixes provide numerous benefits including, but not limited to, enhancing soil micro-flora and fauna, providing soil cover thereby reducing soil erosion, and offering other ecosystem services such as pollination resources (Rodriguez et al., 2009), soil carbon sequestration (Lange et al., 2015), interrupting a pest or disease cycle (Blanco-Canqui et al., 2015), and biodiversity maintenance (Isbell et al., 2017).

Legumes added to the mix of plant species used as forage mixes have the potential to enhance available nitrogen by fixing atmospheric nitrogen, lowering nitrate-leaching losses, and boosting nitrogen release (mineralization) (Finney and Kaye, 2016). In a management-induced fertility gradient research conducted by Schipanski and Drinkwater (2011), red clover introduced to a mix of different cultivars of wheat grown conventionally in New York fixed a total of 57 kg N ha⁻¹ at the end of the first year. Pirhofer-Walzl et al. (2012) found that red clover, white clover (*Trifolium repens* L.), and alfalfa contributed 40 kg N ha⁻¹ to other forage species in a mix, demonstrating yet another grass-legume interaction with the environment. Chicory (*Cichorium intybus* L), plantain (*Plantago lanceolata* L), salad burnet (*Sanguisorba minor* L.), caraway (*Carum carvi* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) all benefited from this interaction,

as they all had an increase in production, which correlates with increased soil cover and reduced soil leaching (Pirhofer-Walzl et al. 2012, Finney and Kaye 2016).

Root biomass in annual forage mixes absorb soil carbon which increases soil particles aggregation, protects organic matter from microbial decomposition, or integrates newly fixed carbon into the existing soil carbon pool (Cong et al., 2014). The quantity of carbon retained in soil is determined by the balance of plant shoot and root debris formation, as well as root organic residues associated with microorganism's breakdown (De Deyn et al., 2011). As a result, the observed increase in soil carbon storage with annual forage mixes is either attributable to increased plant productivity or to the longer persistence of plant-derived organic compounds in the soil (Lange et al., 2015).

2.7. Economic and Profitability of Annual Forages and Forage Mixes

Pasture-raised dairy farmers face pressure to make economic decisions that increase production efficiency and profit while reducing nutrient losses to the environment and adhering to environmental regulation (Rotz et al., 2018). Furthermore, factors such as weather, feed costs, and farm production systems, all have an impact on crop and feeding management (Rotz et al., 2018). Annual forage mixes including grass and legumes produce larger yields and generate better economic returns than legume monocultures and nitrogen-fertilized grass (Sanderson et al., 2018; Sturludóttir et al., 2014; Humphreys et al., 2012).

Butler et al. (2012) observed no differences in average gross revenue between solely planted annual ryegrass fertilized with 112 kg N ha⁻¹ and a grass-legume mix of forage pea, arrowleaf clover (*Trifolium vesiculosum* Savi.), hairy vetch, and annual ryegrass in Oklahoma, both with a gross revenue of \$816 ha⁻¹. However, the three-year cost of production associated with nitrogen application on annual ryegrass was \$570, which was 3% more than the \$551 cost

of production associated with annual ryegrass-legume combination. Furthermore, ADG of 1.07 kg per head d⁻¹ of the grass-legume mixture and the N-fertilized annual ryegrass were identical (Butler et al., 2012). In terms of production cost, annual forage mixes are more advantageous than sole crops.

Farney et al. (2018) looked at the cost, dry matter yield, and composition of forage mixes and found that berseem clover/oat/turnip mixes had the lowest production cost at \$264 Mg⁻¹ DM with a biomass yield of 2.9 Mg ha⁻¹ over two years. The percentage composition of oat in the mix was 78% for the first year and 90% for the second year. In comparison, the least cost-effective mix was ryegrass/radish /berseem clover, which had a production cost of \$683 Mg⁻¹ DM and output of 1.8 Mg ha⁻¹ over two years (Farney et al., 2018). In both years, the botanical composition of ryegrass in the mix was 1%. The cost of creating three-way mixes including brassicas, legumes, and grasses was inversely correlated to the dry matter yield composition of grass species within the mix (Farney et al., 2018).

3. MATERIALS AND METHODS

3.1. Field Experimental Site

The experiments were conducted in Fargo, ND in 2019 and 2020 (46.89639167, -96.08127264 and an elevation of 274 m), near Hickson, ND in 2021 (46.637886, -96.824206, elevation 280 m), and at the Central Grassland Research Center (CGREC) near Streeter, ND in 2021 (46.718014, -99.462056, elevation 607 m) (NDAWN, 2021). The soil types in these sites are Fargo-Ryan, thick silty clays with 18% CaCO₃, 3% gypsum and 0-1% slope for the Fargo site; Fargo-Hegne silty clay, poorly drained, 25% CaCO₃, non-saline to slightly saline with high water storage and 0-1% slope for the Hickson site, and 61% Hecla-Ulen loamy fine sands, low precipitation, 10% CaCO₃, non-saline to very slightly saline with low water storage and 0-6% slope for Streeter (Web Soil Survey, 2020).

Seventeen forage species [annual ryegrass, chicory, plantain, red clover, brassica hybrid (*B. rapa* x *B. oleraceae*), turnip, oat, forage pea, sorghum x sudangrass and sweet sorghum blend, foxtail millet (*Setaria italica* L.), faba bean (*Vicia faba* Roth), forage pearl millet, sorghum x sudangrass, radish, phacelia (*Phacelia tanacetifolia* Benth.), brachytic sorghum BMR, and pearl millet] were included in the experiments in different combinations to create 12 treatments (mixes) (Table 3.1).

Treatment 1 was designed for frequent high-quality grazing; Treatment 2 was intended for fall grazing only; Treatments 3 and 4 were implemented to maximize the nutritive value of forage sorghum cultivars present in the mix; Treatment 5 and 6 were brassica/warm-season annual grass mixes intended to optimize forage nutritive value; and Treatment 7 was composed to enhance biodiversity (Table 3.1).

The experiment was a randomized complete block design with four replicates. Seven out of the 12 treatments were a combination of different species described in Table 3.1. The previous crops grown in the research sites were forage sorghum and kenaf (*Hibiscus cannabinus* L.) in 2019 and 2020, respectively, at Fargo; sugarbeet (*Beta vulgaris* L.) at Hickson, and spring triticale for hay production at the CGREC near Streeter.

Table 3.1. Forage species, cultivar, and pure live seed (PLS) seeding rate in each treatment planted at Fargo, ND, Hickson, MN, and Central Grasslands Research Extension Center near Streeter, ND.

Treatment	Forage crop	Species	Cultivar	Seeding rate PLS kg ha ⁻¹
1	Annual ryegrass	<i>Lolium multiflorum</i> L.	Crusader	13.5
	Chicory	<i>Cichorium intybus</i> L.	Choice	2.2
	Plantain	<i>Plantago lanceolata</i> L.	Tonic	3.3
	Red clover	<i>Trifolium pratense</i> L.	Relish/Emarwan	3.3
2	Hybrid brassica	<i>B. rapa</i> x <i>B. oleraceae</i> L.	Winfred	2.2
	Turnip	<i>Brassica rapa</i> L.	New York	2.2
3	Hybrid brassica	<i>B. rapa</i> x <i>B. oleraceae</i> L.	Winfred	2.2
	Oat	<i>Avena sativa</i> L.	Paul	5.6
	Forage pea	<i>Pisum sativum</i> L.	Arvika	5.6
	Forage sorghum x sudangrass/ sweet sorghum blend	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	Pampa Legion BMR-6 [†]	2.2
	Foxtail millet	<i>Setaria italica</i> var. <i>rubrofructa</i> L.	Siberian	2.2
4	Turnip	<i>B. rapa</i> L.	New York	1.1
	Forage sorghum x sudangrass/ sweet sorghum blend	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	Pampa Tribuno XLT [‡] BMR-6	2.2
	Forage pea	<i>Pisum sativum</i> L.	Arvika	5.6
	Hybrid brassica	<i>B. rapa</i> x <i>B. oleraceae</i> L.	Winfred	1.1
	Oat	<i>Avena sativa</i> L.	Paul	2.2
	Faba bean	<i>Vicia faba</i> Roth.	Sampo	2.2
	Forage pearl millet	<i>Cenchrus americanus</i> (L.) Morrone	Pampa Mijo II BMR-6	2.2
5	Forage pearl millet	<i>C. americanus</i> (L.) Morrone	Pampa Mijo II BMR-6	5.6
	Hybrid brassica	<i>B. rapa</i> x <i>B. oleraceae</i> L.	Winfred	2.2
6	Forage sorghum x sudangrass	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	ADSGS6504 BMR-6, brachytic	2.2
	Radish	<i>Raphanus sativus</i> L.	Graza	2.2
7	Oat	<i>Avena sativa</i> L.	Paul	5.6
	Phacelia	<i>Phacelia tanacetifolia</i> Benth	VNS	1.1
	Forage pea	<i>Pisum sativum</i> L.	Arvika	5.6
	Faba bean	<i>Vicia faba</i> Roth	Sampo	5.6
	Forage sorghum x sudangrass	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	AF7101 BMR-6	3.6
8	Forage sorghum x sudangrass/ sweet sorghum blend	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	Pampa Legion BMR-6	11.2
9	Forage pearl millet	<i>C. americanus</i> (L.) Morrone	Pampa Mijo II BMR-6	11.2
10	Forage pearl millet	<i>C. americanus</i> (L.) Morrone	Pampa Mijo Platino	11.2
11	Forage sorghum x sudangrass	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	AF7101 BMR-6	11.2
12	Forage sorghum x sudangrass	<i>S. bicolor</i> x <i>S. bicolor</i> var. <i>sudanense</i>	ADSGS6504 BMR-6	11.2

[†] Pampa Legion is a physical blend that contains 80% of Pampa Verde (BMR-6 trait, photoperiod sensitive) and 20% of Pampa Karamelo (sweet sorghum cultivar).

[‡] Pampa Tribuno XLT is a physical blend that contains 80% of Pampa Triunfo XLT (BMR-6 trait) and 20% of Pampa Karamelo (sweet sorghum cultivar).

VNS, Variety not stated

3.2. Soil Sampling

Composite soil samples were collected from each replicate at 0-15 cm and 15-60 cm in depth in each site prior to sowing. A total of four composite samples per site were collected. Samples were sent to the soil testing laboratory at North Dakota State University to measure NO₃-N, organic matter, soil pH, phosphorus (P), and potassium (K) in the 0-15 cm soil samples. Only NO₃-N was tested in the 15-60 cm soil samples using the following methods: organic matter, loss on ignition (Schulte and Hopkins, 1996); NO₃-N, colorimetric determination by trans-nitration of salicylic acid (Vendrell and Zupancic, 1990); P Olsen procedure using Brinkmann PC 910 colorimeter (Watanabe and Olsen, 1965); and K, ammonium acetate method using Buck Scientific Model 210 VGP atomic absorption spectrophotometer (Summer and Miller, 1996).

Table 3.2. Nutrient content of soils pre-planting at the Fargo, ND, Hickson, MN, and Central Grasslands Research Extension Center near Streeter, ND.

Site	N-NO ₃ 0-15 cm	N-NO ₃ 15-60 cm	Phosphorus	Potassium	OM	pH
	kg ha ⁻¹		mg kg ⁻¹		g kg ⁻¹	
Fargo 2019	24	47	17	277	60	7.7
Fargo 2020	24	29	15	348	61	7.4
Hickson 2021	22	20	7	271	80	7.5
Streeter 2021	61	65	6	176	37	7.7

OM: organic matter, N-NO₃: Nitrate

Table 3.3. Seeding and harvesting dates for annual forage treatments at the Fargo, ND, Hickson, MN, and Central Grasslands Research Extension Center near Streeter, ND.

Treatment (Trt)	Fargo, 2019	Fargo, 2020	Hickson, 2021	Streeter, 2021
	Seeding date			
All, but Trt 2	03 June	15 May	18 May	25 May
Treatment 2	02 July	01 July	30 June	30 June
	Harvest date			
Harvest Number				
First	01 Aug.	22 July	03 Aug	15 Sept
Second	25 Sept	10 Sept	20 Sept	
Trt 1 only harvest	9 Oct			

Trt 1: Annual ryegrass, chicory, plantain, and red clover; Trt 2: Turnip and hybrid brassica

All experimental plots were 7.62-m long in four rows 0.15-m apart for monocrop, and in eight rows for forage mixes. An 8-cone continuous plot drill (XL Wintersteiger, Salt Lake City, UT) was used for the monoculture and mixes, respectively. For all species, 1000-seed weight is apportioned to calculate the exact number of live seeds needed per row. Pure live seeds were calculated using the germination rate obtained in growth chamber experiments. Seeds were sown at 1-cm depth.

All experiment sites were fertilized with 80 kg N ha⁻¹ and 100 kg P₂O₅ ha⁻¹ after seeding in 2019 and 2020. In 2021, 80 kg N ha⁻¹ and 60 kg P₂O₅ ha⁻¹ were applied to all experimental units. There was no potassium application in any of the experimental sites.

3.3. Sampling and Data Collection

Normalized difference vegetation index (NDVI) was evaluated using a handheld Green-Seeker (Trimble Inc., Sunnyvale, CA) at different growing stages and repeated in the second harvest in 2019, 2020, and 2021. The Green-Seeker was placed above the center-rows of each sampling-plot. The NDVI is a measurement of plant health based on how a plant reflects sun light at specific wavelengths. To be more specific, NDVI is a measurement of the reflectivity of plants expressed as:

$$NDVI = \frac{NIR - VIS}{NIR + VIS}$$

Where, NIR= near-infrared reflectivity and VIS= red reflectivity

Photosynthetically active radiation (PAR) reading below and above the canopy was noted at different growing stages in 2019, 2020, and 2021 by placing a ceptometer (Decagon Devices, Inc. Model LP-80) in between the two-center rows. Three readings were taken from each experimental unit and the ceptometer provided the average readings. Intercepted PAR light percentage was calculated using the following formula:

$$\text{Intercepted PAR light (\%)} = \frac{\text{Light reading above canopy} - \text{Light reading below canopy}}{\text{Light reading above canopy}} \times 100$$

Plant height was measured before each harvest from each experimental unit. Plant height was quantified with a measuring stick from the ground level to top level of the uppermost leaf.

Plant growing stage was recorded during each harvest.

Table 3.4. Normalized difference vegetation index (NDVI) and photosynthetically active radiation (PAR) sampling dates, days from seeding (DAS) and days from first harvest (DAH).

Sampling	Fargo 2019	Fargo 2020	Hickson 2021	Streeter 2021
First	15 July, 42 DAS	13 July, 28 DAS	29 June, 42 DAS	19 July, 45 DAS
Second	24 July, 51 DAS		15 July, 58 DAS	6 Aug, 53 DAS
Third	16 Aug 15 DAH	4 Aug 13 DAH		30 Aug, 77 DAS
Fourth	19 Sept 35 DAH	9 Sept 36 DAH	17 Sept 44 DAH	

The first harvest was completed when the forage sorghum height was 1.5m. The two-center rows of each plot were harvested with a flail-forage harvester (Carter, Brookston, IN) in the first and second harvest, leaving 15-cm stubble for regrowth. Prior to harvest, plants within one-linear meter of the two-center rows were harvested, then weighed fresh and placed in separate bags by species to estimate botanical composition. Samples were dried and weighed to determine botanical composition. However, for the statistical analysis, the botanical composition

was analyzed as the percentage of the most common crop in the mix and the sum of the rest of the species.

$$\% \text{ Botanical composition is calculated as: } \frac{\text{Dry weight of a species}}{\text{Total weight of all species in the mix}} \times 100$$

The second harvest was conducted when forage sorghum reduces its photosynthetic rate due to frost. Growing degree days (GDD) were assessed from minimum and maximum daily temperatures from the North Dakota Agricultural Weather Network (NDAWN, 2021). In this research we considered 15°C as base temperature to calculate GDD for forage sorghum (Thomas and Miller, 1979).

$$\text{GDD} = \sum \left[\frac{(\text{maximum temperature} + \text{minimum temperature})}{2} - \text{Base temperature} \right]$$

Biomass yield was calculated by weighing the total harvested fresh biomass from each plot. Then, a sample of about 1 kg of fresh biomass was dried at 45°C until constant weight. Dry matter content and dry biomass yield was calculated.

A Foss XDS near-infrared instrument (XDS analyzer, Foss, Denmark) was used to scan ground samples throughout a wavelength range of 400–2498 nm. Before scanning, 1.8 g of ground sample was placed in crystal cups with an outer diameter of 5.1 cm and an interior diameter of 3.8 cm, and a disposable back foam board was placed on the back to provide an equal covering of seed across the crystal. Mosaic version 8.4.4.15 software was used to extract the spectrum data, which had a resolution of 2 nm. The calibration equation for the biomass mixes was developed with 93 samples wet chemistry data using near infrared spectra evaluated with WinISI version 4.10.015326 software. Duplicates were averaged once the spectral data was entered into WinISI, and the averages were then utilized to create the calibration equations. Laboratory data on the nutritive value of biomass mixes, known legumes, cool- and warm-season

cultivars, and NIR spectrum data were imported into WinISI and utilized to create a calibration file. For scatter correction, eight alternative pre-spectra processing approaches were utilized. Once the pre-spectra processing was finished, the same techniques were utilized to produce calibrations from each of the pre-spectra processing results. There was a total of 64 equations that were tested. Standard error of cross validation (SECV) and cross validation (1-VR) values were used to compare calibration equation models, and the coefficient of determination was used to assess them (r^2). In addition to the different math treatments, calibration equations were created using modified partial least squares (PLS) regression and four sets of cross validation (Wittenberg et al., 2019).

Dried biomass samples were ground to a 1-mm mesh with a Model 4 cutting mill (Eberbach Corporation, Ann Arbor, MI, USA). Samples of the first harvest in 2019 was sent to the Animal Sciences Laboratory at North Dakota State University for wet chemistry analysis for nitrogen content with Kjeldahl method. Crude protein (CP) was analyzed with the Associate of Official Agricultural Chemists (AOAC) Method 2001.11. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were done using automated dietary fiber analysis apparatus and extraction (ANKOM, 2011. A200 Method). Acid detergent lignin (ADL) and Ash were analyzed with AOAC Method 942.05. Nitrogen accumulation was estimated by multiplying N content in biomass by the dry matter biomass yield.

Total digestible nutrient (TDN) was used as the standard to determine the nutritive value of annual forage mixes. Since TDN encompasses the different properties that affect the nutritive value of forage. To determine TDN, we first derived non-fiber carbohydrate (NFC), which is calculated;

$$\text{NFC} = 100 - \text{CP} - \text{Ash} - \text{EE} - \text{NDF}$$

Where EE is ether extract. All parameters were given by the NIR analyzer (Foss XDS near-infrared instrument, description below). Indigestible neutral detergent fiber was given by the NIR and was used to calculate the digestible neutral detergent fiber (NDFD).

$$\text{NDFD} = 100 - \text{indigestible NDF}$$

TDN was determined using the formula developed by National Research Council (NRC, 2001)

$$\text{TDN} = [(\text{NFC} \times 0.98) + (\text{CP} \times 0.93) + (\text{FA} \times 0.97 \times 2.25) + \left(\text{NDF} \times \left(\frac{\text{NDFD}}{100} \right) - 7 \right)]$$

3.4. Data Analysis

All data samples were analyzed separately by year and location using the procedure MIXED in SAS 9.4 software (SAS Systems Inc., Cary, NC). Homogeneity of variance test was calculated to determine if environments (location/year combination) could be combined. Environments were not homogeneous, so results are presented separate by each environment. Treatment was a fixed effect and environments, and replicates were random effects.

Analysis of variance was conducted using a procedure MIXED of SAS. Least square means pair difference was calculated with the *p-diff* function of MIXED. Treatment mean separation was calculated using the standard error value generated by *p-diff* function to estimate the least significant difference (LSD) value at 95% level of confidence.

3.5. Cost Analysis

Seed price of mixes was calculated using the average cost of retail seed price according to NDSU cover crop calculator multiplied by the seeding rate (Meehan, 2021) (Table 3.5). The cost of all species in a mix was summed to get the total seed cost per mix per ha (Table 3.6).

The cost of seed per dry matter biomass yield was determined by factoring the seed cost per hectare of mix/sole crop in relation to the DM yield of the same mix/sole crop (Farney et al., 2018) (Table 3.6).

$$\text{Seed cost per DM yield} = \frac{\text{total cost of seed mix per hectare}}{\text{DM biomass yield per hectare}}$$

To validate the results, a sensitivity analysis was done to assess variations for each mixture using 10% increase and decrease of total DM yield and of total seed cost of each mix. To achieve this, the mean total DM biomass yield ha⁻¹ of each treatment, averaged across all environments (location/year combination), was calculated.

Table 3.5. Seeding rate and seed cost of annual forage mixes and forage sorghum monocultures in 2021.

Species	Seeding rate [†]	Seed cost per kg	Seed cost per ha	Cultivar/description
	kg ha ⁻¹	\$ kg ⁻¹	\$ ha ⁻¹	
Annual ryegrass	13.5	2.5	34.2	Crusader
Chicory	2.2	11.5	25.7	Choice
Plantain	3.4	11.7	47.6	Tonic
Red clover	3.4	4.2	14.2	Relish/Emarwan
Hybrid brassica	2.2, 1.1‡	7.3	16.4, 7.3	Winfred
Turnip	2.2, 1.1	5.7	12.8, 5.7	New York
Radish	2.2	8.2	12.8	Graza
Oat	2.2, 5.6	0.9	2.1, 5.3	Paul
Forage pea	5.6	0.9	4.9	Arvika
Faba bean	2.2, 5.6	2.4	5.3, 13.3	Sampo
Forage sorghum x sudangrass and sweet sorghum blend	2.2, 11.2	3.9	8.8, 43.9	Pampa Tribuno
Forage pearl millet	11.2	4.2	47.6	Pampa Mijo Platino
Forage pearl millet	2.2, 5.6, 11.2	4.2	9.5, 23.8, 47.6	Pampa Mijo II BMR6
Forage sorghum x sudangrass	2.2, 11.2	1.8	3.9, 19.8	ADSGS6504
Forage sorghum	3.4, 11.2	3.0	10.2, 34.0	AF7101
Phacelia	1.1	14.3	16.0	VNS
Foxtail millet	2.2	2.0	4.4	Siberian

[†]All seeding rates were extracted from NDSU cover crop calculator (Meehan, 2021)

[‡]Species with two or more seeding rates indicates seeding rates for different treatments/ mixes

Table 3.6. Total cost of seed in each annual forage mixture and monocrop in 2021.

Treatments	Cost (\$ ha ⁻¹)
Mix 1	111.6
Mix 2	29.2
Mix 3	39.9
Mix 4	63.1
Mix 5	40.2
Mix 6	25.2
Mix 7	41.8
Monocrop 8	43.9
Monocrop 9	47.6
Monocrop 10	47.6
Monocrop 11	43.9
Monocrop 12	34.0

4. RESULTS AND DISCUSSION

4.1. Climate Conditions

From planting to harvest, Fargo-2019 site (Fig. 4.1A) had the greatest and well-distributed amount of rainfall compared with the study locations. The Fargo-2019, Fargo-2020 (Fig. 4.1B), Hickson-2021, and Streeter-2021 received 440 mm, 326 mm, 328 mm, and 175 mm of rain from sowing to harvest, respectively (NDAWN, 2021). Rainfall in Hickson-2021 and Streeter-2021 was below the 30-year average, with lengthy periods of no rainfall in May, June, and July (Fig. 4.1C and Fig. 4.1D). From sowing to harvest, Streeter-2021 received the least amount of rain (Fig. 4.1D).

The average air temperature was similar across all environments at the time of seeding excluding Streeter-2021 which had the lowest average air temperature (10.2°C) at sowing (Fig. 4.1D). During the growing season, soil temperatures were similar in all environments, but in Fargo-2020 and Streeter-2021 the temperature was below the 15°C-base temperature needed for sorghum the first week after planting. Due to these cooler temperatures and drier growing season (less than normal rainfall of only 0-mm and 4-mm in the week after sowing) after planting at the Fargo-2020 and Streeter-2021 sites, plant emergence was slow, respectively (Fig. 4.1A and Fig. 4.1D). At the end of the season, temperature dropped below 15°C between 7 September and 27 September in all environments (Fig. 4.1 A, B, C and D).

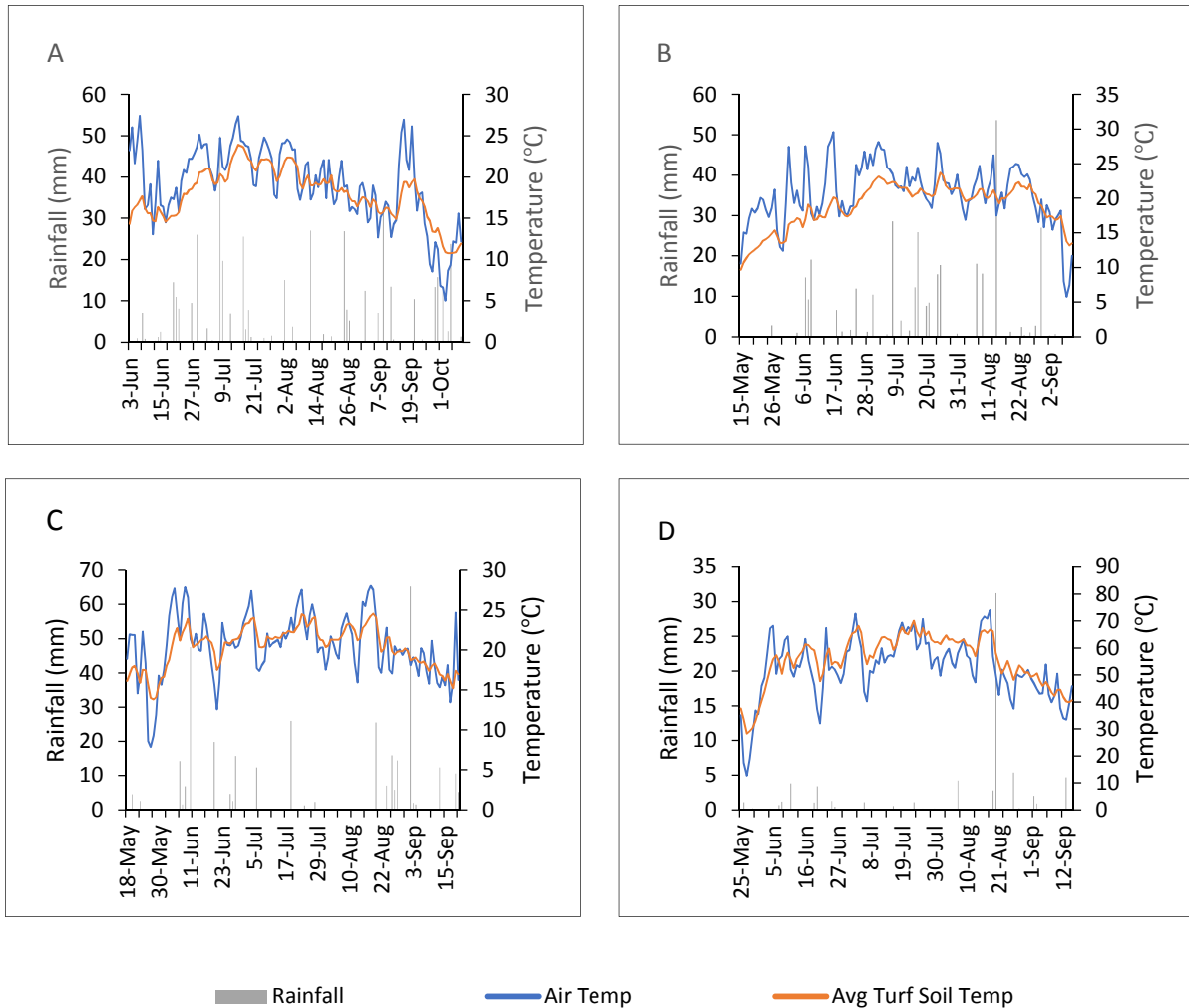


Figure 4.1. Total daily rainfall, and daily average soil and air temperature in Fargo in 2019 (A) and 2020 (B), Hickson in 2021 (C), and Streeter in 2021 (D).

4.2. Normalized Differential Vegetation Index (NDVI)

The analysis of variance for NDVI before the first harvest was significant for the treatment effect at 42 and 51 DAS in Fargo-2019 and at 45 DAS in Streeter-2021 ($p \leq 0.05$) (Table 4.1). After the second harvest, NDVI was significant among treatments ($p \leq 0.05$) at Fargo-2019 at 15 and 35 DAH, at Hickson-2021 at 44 DAH, and at Streeter-2021 at 77 DAS (Table 4.1). There were no differences ($p > 0.05$) among treatments in Fargo-2020 at any of the times NDVI was measured (data not shown).

Table 4.1. Analysis of variance and mean square values of normalized differential vegetation index (NDVI) measured before and after the first and second harvest in annual forage mixes in Fargo, Hickson, and Streeter, ND, in 2019, 2020, and 2021.

		Fargo 2019		Fargo 2020		Hickson 2021		Streeter 2021					
First harvest													
SOV	df	42	51	df	28	-	df	42	df	58	df	45	53
Days after sowing (DAS)													
Rep	3	0.0006	0.0029	3	0.0007	-	3	0.0018	3	0.0013	3	0.0283*	0.0003
Treatment	10	0.0041*	0.0006*	10	0.0005	-	11	0.0027	10	0.0006	9	0.0219*	0.0016
Error	30	0.0011	0.0005	30	0.0003	-	28	0.0024	30	0.0008	27	0.0066	0.0013
CV, %		4.4769	2.9579		2.2442	-		7.4059		3.8882		15.5673	5.2218
Second harvest													
Days after harvest (DAH)													
DAS													
		15	35	13	36			44			77		
Rep	3	0.0087	0.0096*	3	0.0004	0.0005	3	-	-	0.0016	3	0.0002	-
Treatment	11	0.0292*	0.0054*	10	0.0009	0.0003	11	-	-	0.0391*	10	0.0041*	-
Error	33	0.0039	0.0013	30	0.0006	0.0003	33	-	-	0.0025	30	0.0008	-
CV, %		9.7849	5.1927		3.4339	2.3334		-		6.9749		4.0659	-

*Significant at $P \leq 0.05$ levels of probability

Excluding Treatments 1 and 3 at 42 DAS at Fargo-2019, monocultures (Treatments 8 – 12) provided more NDVI ($p \leq 0.05$) than forage mixes (Treatments 4 – 7) (Fig. 4.2A). Mix 7 had the lowest NDVI index of 0.7 at 51 DAS compared with all other treatments ($p \leq 0.05$) (Fig. 4.2A). After the first harvest in Fargo-2019 at 15 DAH, Treatments 3, 7, and Treatment 11 (monocrop) had a NDVI of 0.57, 0.54, and 0.50, respectively and lower than the other treatments ($p \leq 0.05$) (Fig. 4.2B). Treatment 1 provided the greatest soil cover after the first harvest at 15 DAH (0.77) and 35 DAH (0.79) ($p \leq 0.05$) (Fig. 4.2B).

The NDVI of monocrops (Treatment 8 -12) and mixes (Treatments 3 - 7) was different in Streeter-2021 at 45 DAS ($p \leq 0.05$) (Fig. 4.2C). However, all treatments had the same NDVI except Treatment 2 at 77 DAS in Streeter-2021 (Fig 4.2C). Similarly, in Hickson-2021 at 44 DAH, the NDVI for Treatment 2 was much lower than all other treatments (Fig. 4.2D).

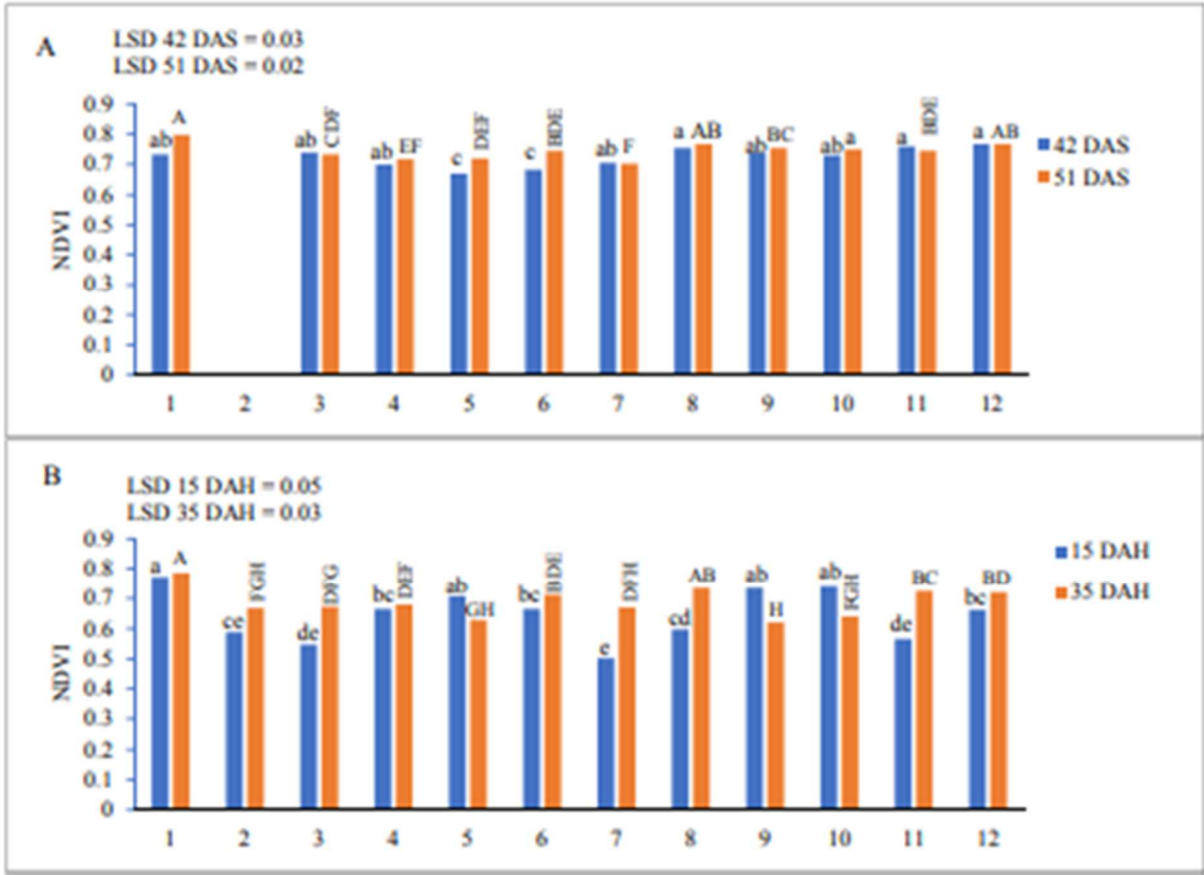


Figure 4.2. Normalized differential vegetative index (NDVI) of annual forage mixes measured in days after sowing (DAS) or days after first harvest (DAH). A) Fargo 2019, B) Fargo 2019, C) Streeter 2021, and D) Hickson 2021; Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudan and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 - 12); 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

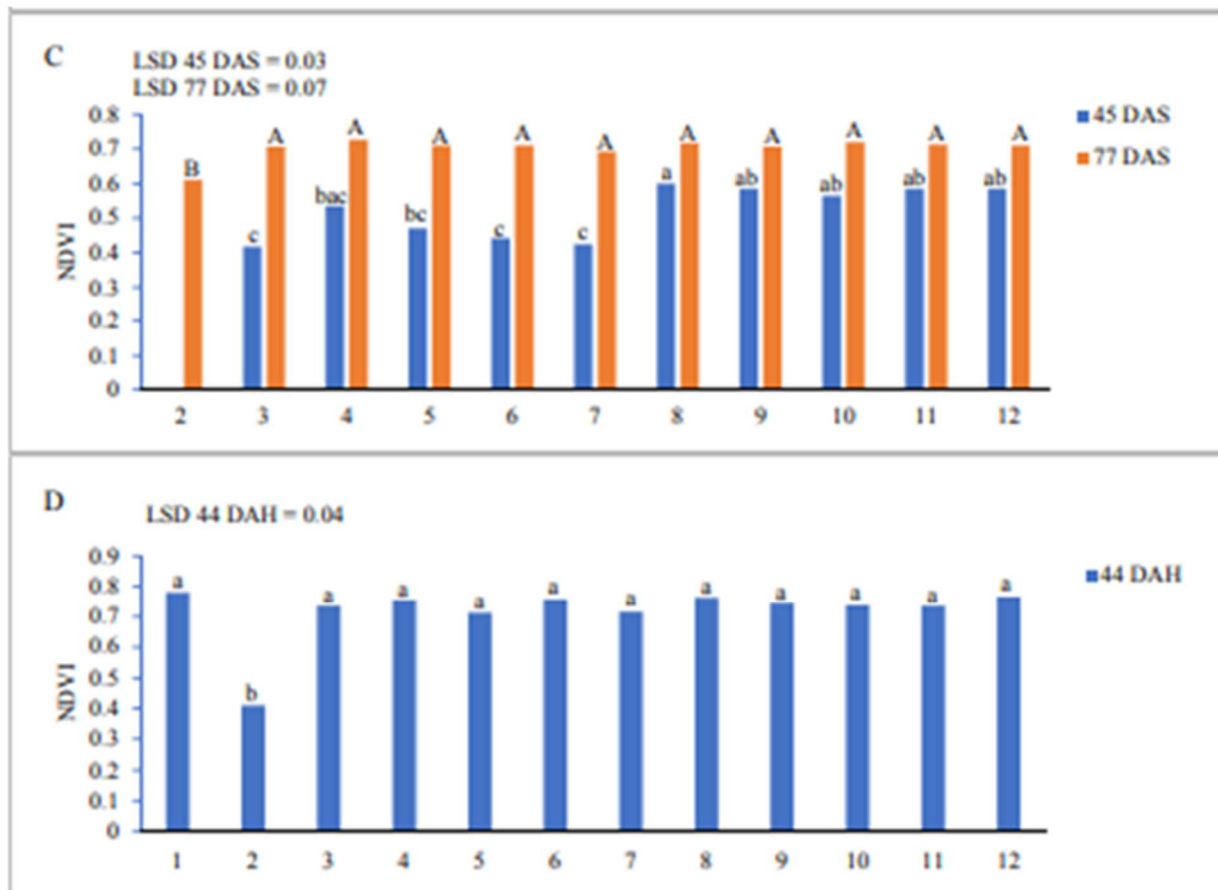


Figure 4.2. Normalized differential vegetative index (NDVI) of annual forage mixes measured in days after sowing (DAS) or days after first harvest (DAH) (continued). A) Fargo 2019, B) Fargo 2019, C) Streeter 2021, and D) Hickson 2021; Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudan and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 - 12); 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

Forage sorghum monocultures or physical blends provided greater soil cover than diverse mixes during early developmental stages, indicating forage sorghum and forage pearl millet growth was faster than that of mixes, perhaps due to reduced early competition for light within treatments in monocrops compared with mixes. Snap et al. (2005) reported the possibility of antagonistic interaction and competitiveness among diverse crop mixes. Due to its ability as a C₄ crop to fix CO₂ at high temperatures in the northern summer of the United States, forage sorghum x sudangrass dominated cowpea [*Vigna unguiculata* (L.) Walp.] in a legume/grass mix in Michigan, producing a biomass yield of 28 Mg ha⁻¹, whereas cowpea only produced 4 Mg ha⁻¹ in the mix (Snap et al., 2005).

The less soil cover of the mix containing oat, phacelia, forage pea, faba bean, and forage pearl millet in our study can be explained because this mix was intended to increase biodiversity and nutritive value rather than fast growth for high biomass yield. In addition, some of these species might be sensitive to competition for light, slowing down their growth rate. In a study conducted in Austria, annual legumes like faba bean and pea were less competitive for light, especially in grass-legume combinations with tall cereal species such as oat that shaded legumes by blocking direct solar radiation access (Neugschwandtner and Kaul, 2014).

Canopy cover of the mix of annual ryegrass, chicory, plantain, and red clover developed slowly at first, but it provided greater cover than all other treatments after the first harvest. Grazing mixes of plantain, chicory, and ryegrass are often utilized in New Zealand. This mix is often grazed every 20-30 days to stimulate rapid regrowth. They take time to establish, but after they are grazed more frequently, growth rate increases and soil cover is greater (Al-Marashdeh et al., 2021). Thus, the delayed soil cover observed at the start of the season followed by full covering towards the conclusion of the season is an expected response (Al-Marashdeh et al.,

2021). Treatment 2, which included solely a hybrid brassica and a turnip, was projected to have less soil cover after the first harvest since it was planted late. Forage brassicas are typically planted late in the summer to provide soil cover and late-fall forage from October through December (Denman et al., 2021).

4.3. Photosynthetically Active Radiation (PAR)

The analysis of variance was significant ($p \leq 0.05$) for intercepted PAR in the first harvest for all measurement dates at all environments, except in Streeter-2021 (Table 4.2). After the second harvest, the intercepted PAR was different ($p \leq 0.05$) among treatments in all environments and measurement dates with the exception of 36 DAH in Fargo-2020 and at 77 DAS in Hickson 2021 (Table 4.2).

Prior to the first harvest at 42 DAS and 51 DAS in Fargo-2019, there was no difference ($p > 0.05$) in PAR among treatments excluding Treatment 2 (Fig. 4.3A). At 15 DAH, Treatment 7 intercepted 37.5% of PAR, the lowest of all treatments, although at 35 DAH there were no differences among treatments, except for Treatment 2 (Fig. 4.3B). Monocrops intercepted more PAR ($p \leq 0.05$) than Treatment 5 and 6 at 28 DAS in Fargo-2020 (Fig. 4.3C). Treatment 1 had the highest intercepted PAR of 76.4% ($p \leq 0.05$) at 13 DAH in Fargo-2020 (Fig. 4.3C). Treatment 7 intercepted the least PAR of 47.5% and was different ($p \leq 0.05$) from Treatments 1, 10, and 8 which intercepted 79.7, 74.7 and 74.0% of PAR, respectively at 36 DAH (Fig. 4.3C).

In Hickson-2021, aside from Treatment 1, monocrops (Treatment 8 -12) intercepted significantly higher PAR than all other mixes (Treatment 2-7) at 42 DAS. However, at 58 DAS, in Treatments 1 and 2 the PAR interception was lower than all other treatments (Fig. 4.3D). After the first harvest, there was no difference among treatments except for Treatment 2 (Fig. 4.3D). In Streeter-2021, at 45 DAS, Treatment 5 intercepted the least PAR of only 12.7% ($p \leq$

0.05). At 53 DAS, Treatments 11, 9, and 10 intercepted PAR was 67.4, 63.9, and 59.2%, respectively and were significantly higher than Treatment 7 ($p \leq 0.05$) with an intercepted PAR of 25.9% (Fig. 4.3E). Treatment 7 intercepted the least PAR ($p \leq 0.05$) in Streeter-2021 at 77 DAS (Fig. 4.3E).

In most environments, the oat/phacelia/forage pea/forage sorghum x sudangrass mix (Treatment 7) intercepted the least PAR after the first harvest, possibly due to the morphological structure of the plant species in the mix. Annual legumes such as faba bean and forage pea did not regrow after the first harvest, leaving open patches of uncovered soil. Cool-season legumes such as faba bean and forage pea, do not grow well at hot temperatures ($> 25^{\circ}\text{C}$) of mid-summer, which inhibits growth and prompts the plant to go into reproductive phase (Sheaffer et al., 2020). Furthermore, forage sorghum is known to secrete allelopathic substances that limit the development of neighboring plants which could have been part of the reason of limited growth of other crops in the mix (Besancon et al., 2021; Dayan et al., 2010).

In contrast to Treatment 7, annual ryegrass/chicory/plantain/red clover mix (Treatment 1) complemented each other well in PAR interception. Annual ryegrass, which is a fast-growing grass, covered the soil quickly, but its narrow, upright leaves likely allowed light to penetrate the canopy without disrupting the growth of upright broadleaves of chicory and plantain (Chatterjee and Clay, 2016). Red clover did not compete well in this mix. After the first harvest, no treatment reached the same PAR interception observed before the initial harvest in both Fargo environments, indicating the time left in the season was not enough to get a complete full canopy (Zhang, 2019). In Streeter-2021, Treatment 1 was harvested only once due to the drought which inhibited growth.

The greater intercepted PAR of forage sorghum monocrops compared with other crops could be the reason for its high biomass productivity. Podder et al. (2019) reported that forage sorghum genotypes intercepted 81% of the PAR at 30 DAH and had an average biomass yield of 18 Mg DM ha⁻¹. The observed difference between Podder et al. (2019) forage-sorghum intercepted PAR and forage sorghum x sudangrass/sweet-sorghum-blend in our study with 74.7% intercepted PAR, might be due to differences in weather and planting date even though both studies were conducted at the same location. The intercepted PAR is influenced by the interplay of the crop's morphology, leaf area index, location, and climate (Kukul and Irmak, 2019). The architectural difference among species changes the amount of PAR intercepted at the maximum leaf area index (LAI), for example the research focused on light interaction use and efficiency of row crop canopies under optimal growth conditions showed that the average intercepted PAR at peak LAI was 73% in soybean, 88% in sorghum 71% in maize, and 50% in wheat at Nebraska. (Kukul and Irmak, 2019).

Table 4.2. Analysis of variance and mean square values for intercepted photosynthetically active radiation (PAR) in Fargo, Hickson, and Streeter, ND, in 2019, 2020, and 2021.

Source	Fargo 2019			Fargo 2020			Hickson 2021			Streeter 2021		
	df	42 DAS	51 DAS	df	28 DAS		df	42 DAS	58 DAS	df	45 DAS	53 DAS
First harvest												
Rep	3	332*	283*	3	120*		3	258*	292*	3	822*	2496*
Treatment	11	1995*	574*	10	213*		11	1932*	1998*	9	986	706
Error	29	50	70	30	34		29	75	65	27	304	491
CV, %		10	9		6			14	11		45	45
Second harvest												
		15 DAH	35 DAH		13 DAH	36 DAH		44 DAH		77 DAS		
Rep	3	122	101*	3	518*	294	3	44	3	1743*	-	
Treatment	11	829*	2630*	10	594*	377	11	356*	9	4950	-	
Error	29	79	23	30	165	196	33	36	27	952	-	
CV, %		14	5		26	21		6		57	-	

*Significant at $P \leq 0.05$ levels of probability

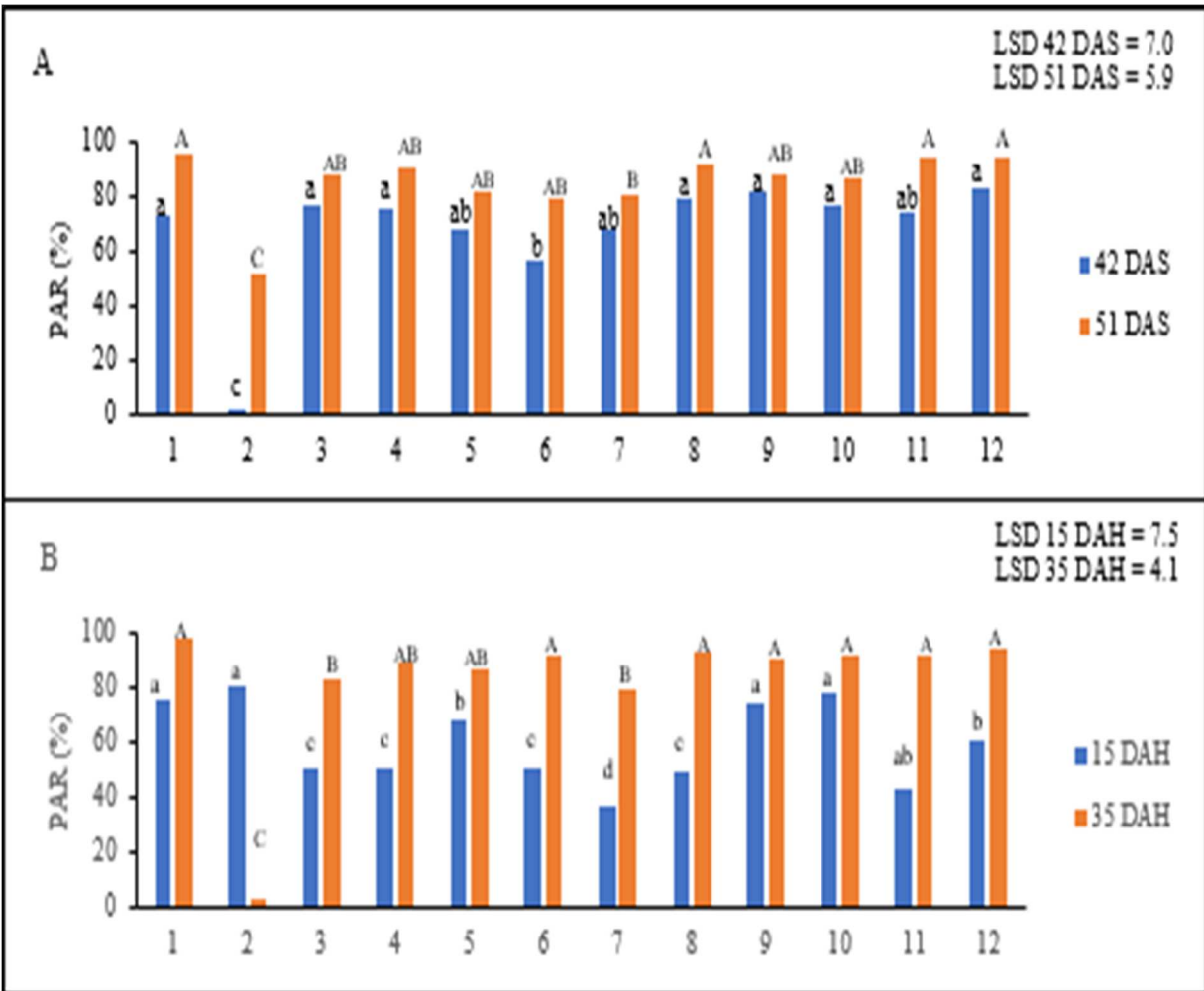


Figure 4.3. Intercepted photosynthetically active radiation (PAR) measured in days after sowing (DAS) or days after the first harvest (DAH): A) Fargo 2019, B) Fargo 2019, C) Fargo 2020, D) Hickson 2021, and E) Streeter 2021. Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudan and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters within a column of same color indicate significant difference ($p \leq 0.05$).

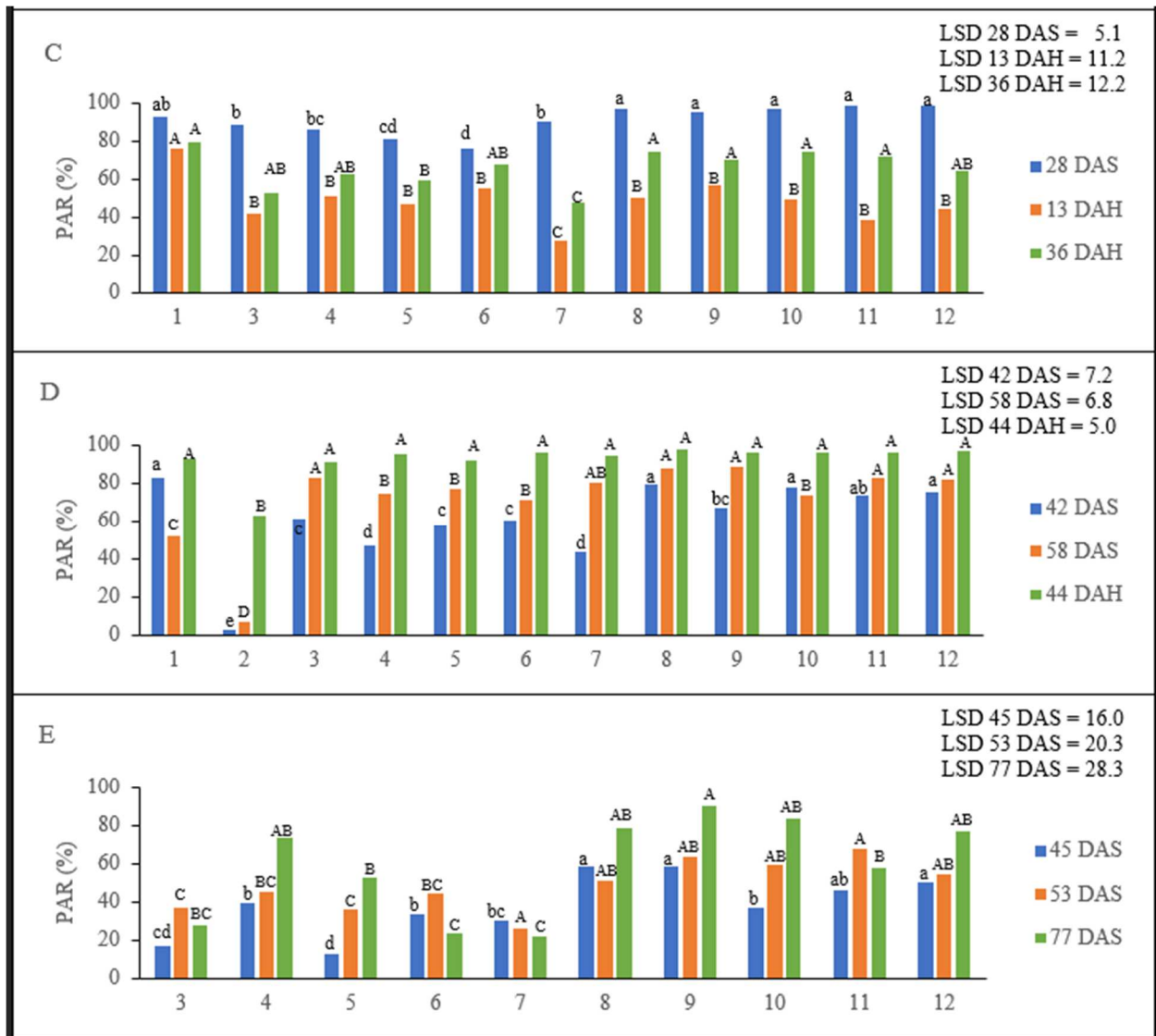


Figure 4.3. Intercepted photosynthetically active radiation (PAR) measured in days after sowing (DAS) or days after the first harvest (DAH) (continued): A) Fargo 2019, B) Fargo 2019, C) Fargo 2020, D) Hickson 2021, and E) Streeter 2021. Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudan and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters within a column of same color indicate significant difference ($p \leq 0.05$)

4.4. Plant Height

The analysis of variance indicated significant differences among mixes across both harvests at all environments, except in Streeter-2021 (Table 4.3). The harvest main effect was also significant in Fargo-2019, Fargo-2020 and in Hickson-2021. A significant interaction between treatment and harvest was observed only in Hickson-2021 (Table 4.3).

Plants in Treatments 8, 11, and 12 were much taller than the plants in other treatments in Fargo-2019, with heights of 1.73, 1.90, and 2.10 m, respectively (Table 4.4). In Fargo-2020, the plants in Treatment 12 were by far the tallest. (Table 4.4). Plants in Treatments 11 and 12 were substantially taller than the plants in other treatments in Hickson-2021. In all sites, Treatment 1 plants were the shortest ($p \leq 0.05$) (Table 4.4).

At the first and second harvest in Hickson-2021, plant height of Treatment 1 plants was 0.38 and 0.55 m, respectively, and they were the shortest of all treatments ($p \leq 0.05$) (Table 4.5). Treatments 11 and 12 were considerably the tallest plants for both harvests ($p \leq 0.05$) (Table 4.5). There was a decline in average plant height between the first and second harvest, with the exception of plants in Treatments 1 and 2 (Table 4.5).

The shortest plants at all environments were the annual ryegrass/chicory/plantain/red clover mix (Treatment 1), which is related to the nature of the species in the mix, as each individual species in Treatment 1 are short crops intended mainly for frequent grazing (Waghorn and Clark, 2004). Plants were shorter in treatments without forage sorghum x sudangrass than in treatments including forage sorghum x sudangrass. This might have had an impact on the treatment's productivity, as a prior study conducted in China indicates that plant height correlates with dry matter biomass productivity (Zhao et al., 2009).

Table 4.3. Analysis of variance and mean square values of annual forages plant height at harvest in Fargo, Hickson, and Streeter in 2019, 2020, and 2021.

Source	Fargo 2019		Fargo 2020		Hickson 2021		Streeter 2021	
	df	height	df	height	df	height	df	height
Rep	3	0.37*	3	0.13*	3	0.24	3	0.11
Treatment	11	1.85*	11	0.75*	11	1.02*	10	0.46
Harvest	1	1.86*	1	0.75*	1	3.01*	.	.
Treatment x harvest	10	0.04	11	0.02	11	0.25*	.	.
Error	66	0.05	63	0.02	64	0.05	29	0.25
CV, %		18.3		44.1		21.2		42.8

*Significant at $P \leq 0.05$ levels of probability

Table 4.4. Mean plant height averaged across two harvests for 12 annual forage treatments in Fargo, Hickson, and Streeter in 2019, 2020 and 2021.

Treatment	Crop	Fargo 2019	Fargo 2020	Hickson 2021
		-----m-----		
1	Annual ryegrass, chicory, plantain, red clover	0.5	0.3	0.5
2	hybrid brassica, turnip			
3	Hybrid brassica, oat, forage pea, forage sorghum x sudangrass/ sweet sorghum blend, foxtail millet	1.2	0.7	1.3
4	Turnip, forage sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, forage pearl millet	0.9	0.6	1.4
5	Forage pearl millet, hybrid brassica	0.8	0.5	1.3
6	Sorghum x sudan, radish	1.1	0.7	1.6
7	oat, phacelia, forage pea, faba bean, forage sorghum	1.1	0.7	1.5
8	Sorghum x sudangrass/ sweet sorghum blend	1.7	1.1	1.6
9	Forage pearl millet	1.1	0.7	0.9
10	Forage pearl millet	1.0	0.7	1.1
11	Forage sorghum x sudangrass	1.9	1.2	1.7
12	Forage sorghum x sudangrass	2.1	1.3	1.6
LSD (0.05)		0.2	0.1	0.2

Table 4.5. Mean plant height for forage mixtures harvested twice in Hickson in 2021.

Treatment	Crop	First harvest	Second harvest
		-----m-----	
1	Annual ryegrass, chicory, plantain, red clover	0.4	0.6
2	Hybrid brassica, turnip		1.1
3	Hybrid brassica, oat, forage pea, sorghum x sudangrass/ sweet sorghum blend, foxtail millet	1.6	1.0
4	Turnip, sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, forage pearl millet	1.6	1.2
5	Forage pearl millet, hybrid brassica	1.3	1.2
6	Sorghum x sudan, radish	1.8	1.4
7	Oat, phacelia, forage pea, faba bean, forage sorghum	1.7	1.4
8	Sorghum x sudangrass/ sweet sorghum blend	2.0	1.2
9	Forage pearl millet	0.9	0.9
10	Forage pearl millet	1.2	1.1
11	Forage sorghum x sudangrass	2.1	1.3
12	Sorghum x sudan	2.0	1.3
LSD (0.05)		0.2	0.2

4.5. Proportion of Forage Sorghum in Mixes with Sorghum

The analysis of variance indicated the main effect of treatment was significant only in Fargo-2019 ($p \leq 0.05$) (Table 4.6). The main effect of harvest was significant in both Fargo-2019 and Fargo-2020 while the interaction between treatment and harvest was significant in Fargo-2019 and Hickson-2021 ($p \leq 0.05$).

Table 4.6. Analysis of variance and mean squares for the botanical composition of the main species (forage sorghum) compared with the total of the other species in the mix in Fargo, Hickson, and Streeter, ND, in 2019, 2020, and 2021.

Source	Botanical composition					
	df	Fargo 2019	Fargo 2020	Hickson 2021	df	Streeter 2021
Rep	3	42	4402*	124	3	113
Treatment	4	4876*	1748	2038	4	1713
Harvest	1	2718*	3644*	3748	.	.
Treatment x harvest	4	1681*	1547	1687*	.	.
Error	27	135	681	164	12	351
CV, sorghum, %		18	50	15		27
CV, other species, %		32	55	75		56

*Significant at $p \leq 0.05$ levels of probability

In Fargo-2019, the botanical composition in mixes having more than two species in the first harvest, Treatments 3, 4, and 7, was 11.9, 41.7, and 38.1% of forage sorghum, respectively, which was considerably less than the monocultures or blends as expected (Fig. 4.4A). In mixes with two species (Treatments 4 and 5), the presence of the other species in the mix was much lower than that of forage sorghum. Forage sorghum dominated all treatments in the second harvest in Fargo-2019, apart from Treatment 4 (Fig. 4.4A).

Similar result was observed in Fargo-2020 where forage sorghum presence in multiple-species mixes was lower than in the dual-species mixes in the first harvest ($p \leq 0.05$) (Fig. 4.4B). Except for Treatment 4, species other than forage sorghum composed 66.1% of the mix in Fargo-2020. As in Fargo-2019, forage sorghum dominated all mixes in the second harvest (Fig. 4.4B).

This trend was also observed in Hickson-2021, although forage sorghum presence in mixes was considerably greater than the other species in Treatment 4 at second harvest (Fig. 4.4C).

Forage sorghum competed successfully with other species in the mix (Fig. 4.4), dominating the mix in the second harvest. This is remarkable considering the forage sorghum-seeding rate was only 2.2 kg ha⁻¹ (Table 3.1), when the recommended seeding rate of forage sorghum in a mix is 4 to 8 kg ha⁻¹ (Snider et al., 2012). Although sorghum did not dominate Treatment 4 in the Fargo environments, this was most likely due to the competition imposed by forage pearl millet, which is also a high-yielding warm-season annual forage. In general, high yielding warm-season grasses influenced the botanical makeup of annual forage mixes in this study. This is comparable to Mercier et al. (2021), who reported forage sorghum and forage pearl millet overshadowed the other annual forage species composing the mix, limiting their growth.

Temperatures above 25°C, which is termed high temperature, were consistent in each study location prior to the first harvest (Fig 4.1). This had an impact on the botanical makeup of mixes in the second harvest since annual cool-season legumes (forage pea and faba bean) and oat (cool-season grass) do not compete effectively under such temperatures. Annual cool-season legumes such as field pea and clovers thrived in temperatures ranging from 10°C to 25°C, with the highest biomass of field pea recorded at 20°C (Butler et al., 2014). Cool-season grasses such as oat excelled in temperatures ranging from 10°C to 20°C, and annual ryegrass grew the fastest in temperatures ranging from 10°C to 30°C. (Butler et al., 2017). In our study, by the time the temperature dropped in the fall, it was too late for cool-season species to resume growth since the first harvest impact and stress from competition had reduced their chances of competing with the already established annual warm-season grasses.

Early planting and stand establishment are critical for forage brassicas production in low rainfall environments (Brill et al., 2016). Brassicas in mixes were slow to germinate due to inadequate rainfall (<10 mm at 20 DAS) in some of the environments, in the early days after sowing; this delayed development lowered their productivity in the first harvest, but its productivity increased in the second harvest. Forage sorghum's capacity to adjust to shifting water availability as a C4 plant gave it the potential to dominate mixes in which it was present (Khan and McVay, 2019).

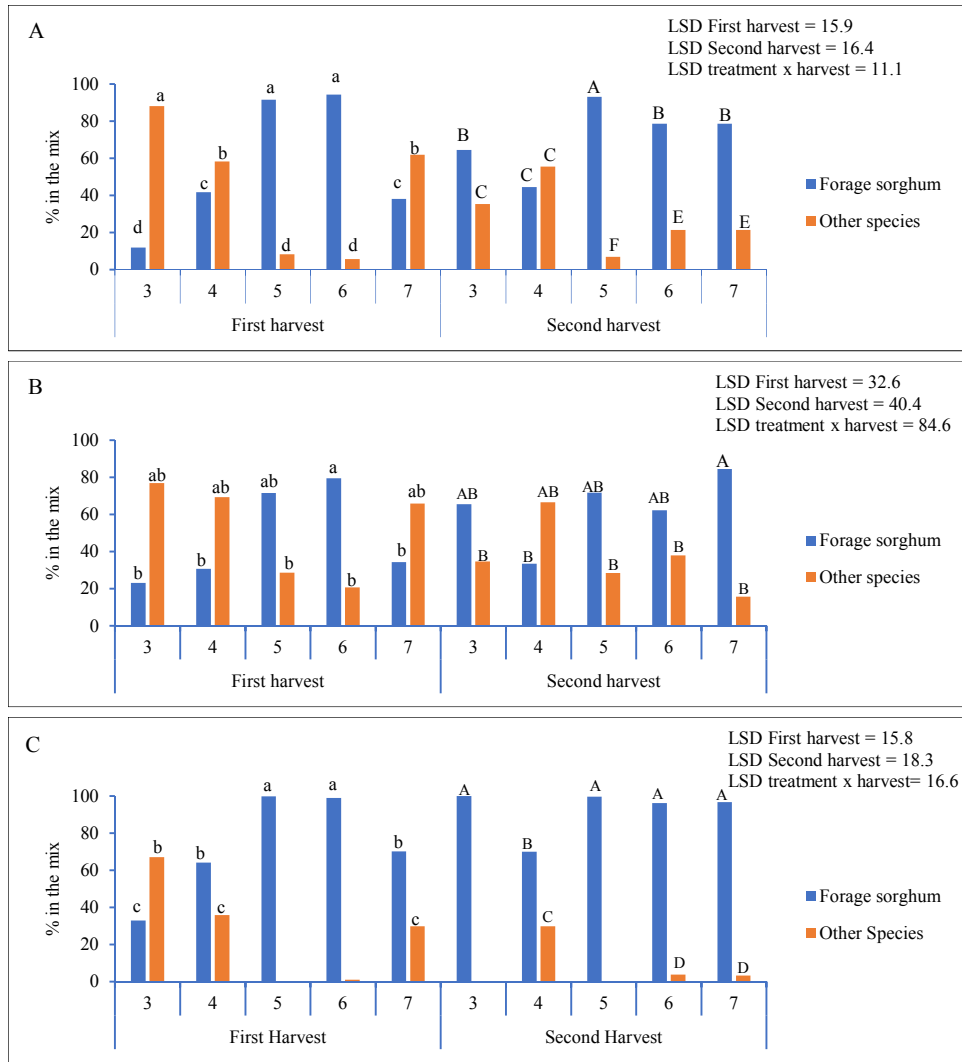


Fig. 4.4. Botanical composition of 12 annual forages in the first and second harvest: A) Fargo 2019, B) Fargo 2020, and C) Hickson 2021. Forage mixes (3 – 7); 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; and 7) oat, phacelia, forage pea, faba bean, and forage sorghum. In each figure, different letters within a same harvest and treatment are significantly different ($P \leq 0.05$).

4.6. Biomass Yield

The analysis of variance indicated treatment, harvest, and their interaction were significant at all locations. Only one harvest was conducted at the Streeter-2021 location due to lack of growing season moisture (Table 4.7). In Fargo-2019, the hybrid brassica/oat/forage pea/forage sorghum x sudangrass x sweet sorghum blend/foxtail millet mixture (Treatment 3) had the highest average production (4.2 Mg ha⁻¹) at the first harvest (Fig. 4.5A). Treatment 3, along with the annual ryegrass/chicory/plantain/red clover mix (Treatment 1) and the hybrid brassica/turnip mix (Treatment 2), yielded less ($p \leq 0.05$) in the second harvest than the other treatments (Fig. 4.5A). With the exception of Treatments 1 and 2, there was no difference in the overall average biomass yield of monocrops and mixes across both harvests in Fargo-2019 (Fig. 4.5A).

The forage sorghum x sudangrass/sweet sorghum blend (Treatment 8), forage sorghum x sudangrass (Treatment 11), and forage sorghum x sudangrass (Treatment 12) produced an average of 8.2 Mg ha⁻¹, 8.5 Mg ha⁻¹, and 8.1 Mg ha⁻¹ of DM biomass, respectively, compared to the other treatments for the first harvest at the Fargo-2020 location (Fig. 4.5B). Aside from the 6.6 Mg ha⁻¹ biomass yield in the sorghum x sudangrass/radish combination (Treatment 6), monocrops (Treatments 8-12) generated significantly greater biomass than mixes (Treatments 1-7) during the second harvest (Fig. 4.5b). In Fargo-2020, monocrops equally averaged a much greater biomass yield ($p \leq 0.05$) in both harvests (Fig. 4.5B).

During the first harvest in Hickson-2021, Treatments 8, 11, and 12 produced ($p \leq 0.05$) greater biomass at 9.3 Mg ha⁻¹, 8.5 Mg ha⁻¹, and 8.5 Mg ha⁻¹, respectively, than the other treatments compared with the previous year at the Fargo-2020 location (Fig 4.5C). Except for Treatment 1 and 2, which yielded 2.3 Mg ha⁻¹ and 0.5 Mg ha⁻¹ respectively, there was no change

in biomass output between treatments in Hickson-2021 in the second harvest compared to the monocrops (Treatment 8-12) (Fig. 4.5C). Treatment 8 had the greatest total average biomass yield of 15.1 Mg ha⁻¹ ($p \leq 0.05$) with both harvests summed up in Hickson-2021 (Fig. 4.5C).

Treatment 2 (0.5 Mg ha⁻¹) produced the least biomass ($p \leq 0.05$) among treatments at the Streeter-2021 location (Fig 4.5D). Treatments 4 and 6, had 2.6 Mg ha⁻¹ and 3.0 Mg ha⁻¹, respectively, with the maximum biomass output for mixes, which was similar to the overall average biomass yield of monocrops at the Streeter-2021 location (Fig 4.5D).

Table 4.7. Analysis of variance and mean squares of biomass yield of annual forage mixes using two harvests at Fargo, ND and Hickson, MN; d and one harvest at Streeter, ND.

Source	Fargo 2019		Fargo 2020		Hickson 2021		Streeter 2021	
	df	Yield	df	Yield	df	Yield	df	Yield
Rep	3	4.3*	3	4.6*	3	10.3*	3	5.2*
Treatment	11	2.5*	10	28.4*	11	19.4*	10	3.8*
Harvest	1	39.4*	1	29.7*	1	65.5*	.	.
Treatment x harvest	10	4.5*	10	9.7*	10	5.0*	.	.
Error	66	0.8	63	1.4	64	1.3	29	1.3
CV, %		27.4		22.9		21		48.9

*Significant at $p \leq 0.05$ levels of probability

The total accumulated growing degree days (AGDD) varied across environments and between harvests. There was at least 155 AGDD difference between the first and second harvest across all locations (Table 4.8). Hickson-2021 had the most AGDD between harvests. Treatment 2 was planted later and harvested only once in all environments, so we report its AGDD separately. Treatment 1 was harvested three times at the Fargo-2019 location, with its resulting yield added to the second harvest. The second harvest was completed at an average daily temperature below 15°C (Table 4.8).

The Hickson-2021 study location had greater biomass yield compared with the other locations, which might be attributed to the highest AGDD (Table 4.8). However, environmental conditions such as rainfall and soil type must be considered. Although the Streeter-2021 site had

more AGDD than Fargo-2019 and Fargo-2020, all treatments produced less biomass, mainly due to below-normal rainfall and less water holding capacity soils.

Table 4.8. Accumulated growing degree days (GDD) with base temperature of 15°C from sowing date to first harvest, and from first harvest to second harvest at each study location.

Environment	Accumulated GDD				
	First Harvest	Second Harvest	Trt 2	Trt 1	Total
Fargo 2019	380	225	380	157	529
Fargo 2020	420	256	432		672
Hickson 2021	456	245	486		695
Streeter 2021	616				616

Trt 1: Annual ryegrass, chicory, plantain and red clover, Trt 2: Hybrid brassica and turnip

Annual ryegrass/chicory/plantain/red clover mix (Treatment 1), while yielding the least of all treatments, produced a minimum of 1.6 Mg ha⁻¹ biomass per harvest at all environments except Streeter-2021 (Fig. 4.5). Treatment 1 production of 1.6 Mg ha⁻¹ (Fig. 4.5) averaged across locations, which would be sufficient feed for 1.5 animal unit month (AUM) ha⁻¹ of 453 kg cow (Andersen et al., 2020).

Despite the fact that each location was unique, there was a consistent trend in the biomass yield of mixes and monocrops. In three of the four locations, monocrops produced greater biomass than mixes, which is attributed to the competitiveness of species within mixes. Forage sorghum and/or pearl millet dominated the mixes at the Fargo-2019 location; hence, there was no difference between the total average yield of monocrop and mixes, with the exception of Treatments 1 and 2.

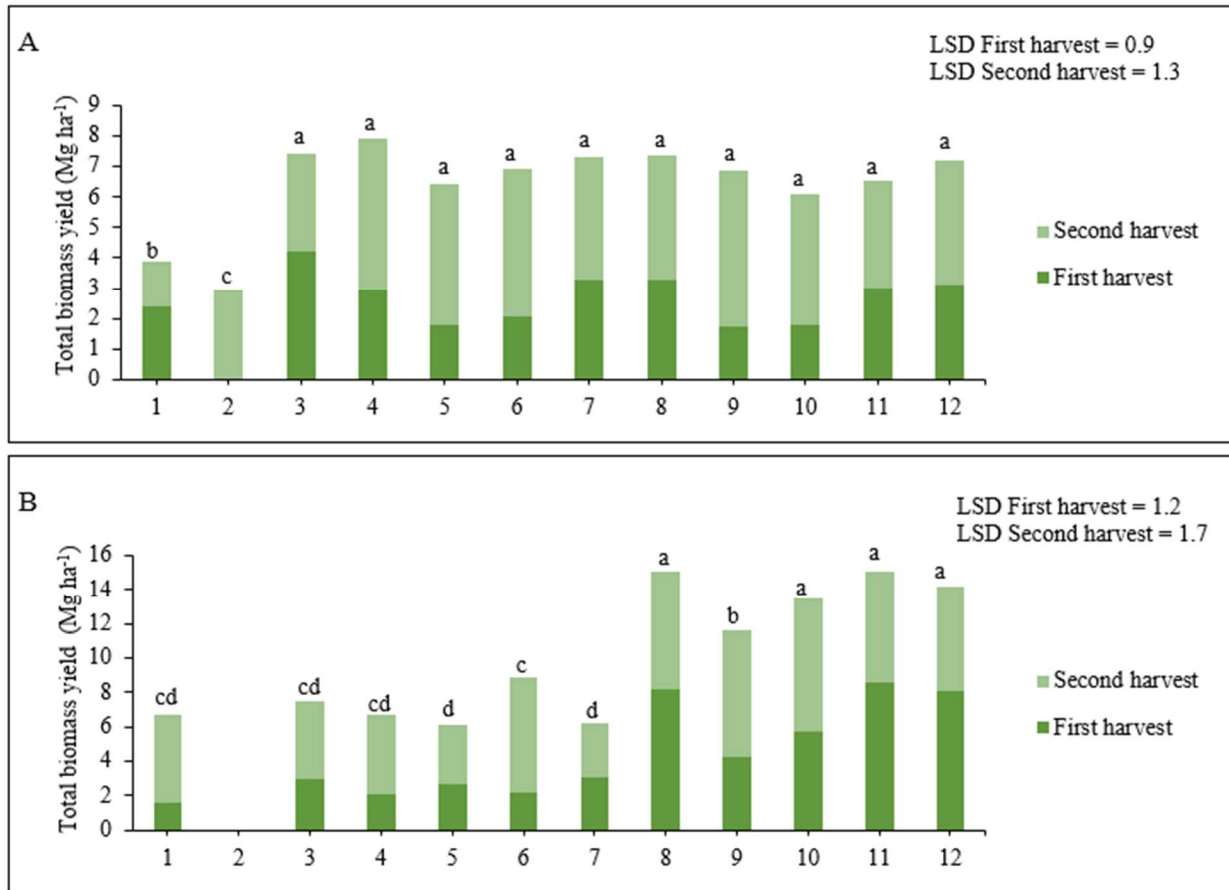


Figure 4.5. Total biomass yield of annual forage mixes and monocrops at Fargo, Hickson, and Streeter, North Dakota in 2019, 2020, and 2021. Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; and 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters indicate significant difference ($p \leq 0.05$).

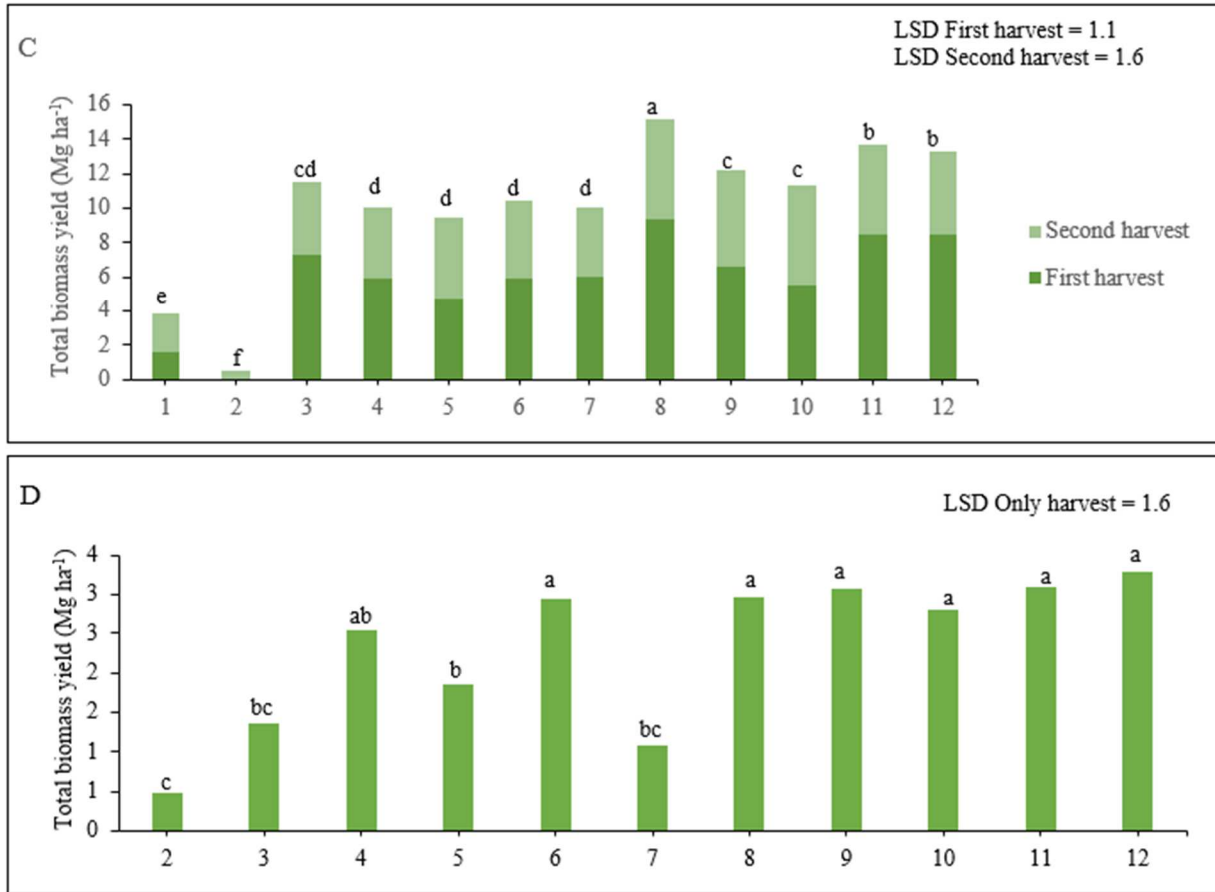


Figure 4.5. Total biomass yield of annual forage mixes and monocrops at Fargo, Hickson, and Streeter, North Dakota in 2019, 2020, and 2021 (continued). Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; and 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In each figure, different letters indicate significant difference ($p \leq 0.05$).

Forage sorghum x sudangrass/sweet sorghum blend total biomass yield was 15.1 Mg ha⁻¹ at both Fargo-2020 and Hickson-2021 at 119 and 126 days from sowing, respectively.

Samarappuli et al. (2014) reported a yield of forage sorghum of 18 Mg ha⁻¹ in 125 days averaged across four environments in North Dakota. The effect of N mineralization from fall-seeded cover crops forage pea and Austrian winter pea (*Pisum sativum spp. arvense* (L.) Poir) could have contributed to a greater biomass output in the Samarappuli et al. (2014) study. However, the differences in rainfall, temperature, and perhaps seeding rate of 23 kg ha⁻¹ should be considered.

Harvest timing may have hampered the possibility of increasing biomass yield in the forage sorghum and forage pearl millet monocrops (Machicek et al., 2019). In our study, there could have been a higher yield in forage sorghum and forage pearl millet if only harvested once at the locations which had two or more harvests. Machicek et al., (2019) reported that after 90 days, BMR forage sorghum x sudangrass and BMR pearl millet yielded almost two times more in one harvest than two 45-day harvests. Forage sorghum x sudangrass/sweet sorghum blend yielded of 15.5 Mg DM ha⁻¹ when both harvests were combined could have been greater if harvested only once. Although variable factors such as forage sorghum type, seeding rate, climate, and weather determines such outcome (Bhattarai et al., 2019).

The biomass yield of mixes without forage sorghum and/or pearl millet were lesser ($p \leq 0.05$) than mixes containing forage sorghum and/or pearl millet, even though the seeding rate of forage sorghum in mixes was only 2.2 kg ha⁻¹. Other researchers have also reported that forage sorghum and pearl millet included in mixes produce more biomass than mixes without it (Hassan et al., 2015).

The forage sorghum sudangrass/radish mix (Treatment 6) yielded more than the other mixes that had more than two species. However, forage sorghum x sudangrass/radish mix was

dominated mainly by forage sorghum. Increasing diversity in annual forage mixes does not increase forage biomass yield compared with monocultures (Bainerd et al., 2020).

The biomass yield of annual mixes was much lower than that of monocrops, contradicting the hypothesis of this study. This was due to the failure for some annual crops to grow due to lack of competition with forage sorghum. Because forage sorghum and pearl millet are tall, high producing biomass crops with little competition when established (Hassan et al., 2015), they can uptake all the nutrients they require to thrive. Furthermore, the allelopathic feature of forage sorghum may have hampered the regrowth of some of the species in the mix, limiting the likelihood of better yield among mixes (Besancon et al., 2021).

4.7. Crude Protein and Total Digestible Nutrients

The analysis of variance indicated crude protein (CP) and total digestible nutrients (TDN) were different among treatments ($p \leq 0.05$) at all locations. The harvest main effect on CP and TDN were significant for all locations except in Streeter-2021, which only had one harvest. The interactions between treatment and harvest effects were significant ($p \leq 0.05$) at all locations except CP at Fargo-2020 and Streeter-2021.

Table 4.9. Analysis of variance and mean squares for crude protein (CP) and total digestible nutrients (TDN) of annual forage mixes harvested twice in the season, in Fargo, Hickson and Streeter, ND, in 2019, 2020, and 2021.

Source	df	Fargo 2019		Fargo 2020		Hickson 2021		Streeter 2021			
		CP	TDN	CP	TDN	df	CP	TDN	df	CP	TDN
Rep	3	12.9*	0.9	20.9*	0.9	3	3.4	0.5	3	1.3	0.8
Treatment	11	17.1*	25.1*	10.7*	12.1*	11	15.4*	7.2*	10	37.9*	5.5*
Harvest	1	91.2*	401.6*	65.5*	294.6*	1	718.2*	32.2*	.	.	.
Treatment x harvest	10	5.5*	3.4*	7.5	12.3*	10	10.6*	3.7*	.	.	.
Error	66	3.1	1.4	5.1	2.3	65	4.3	1.8	30	4.9	1.3
CV, %		14.5	1.7	18.8	2.2		17.3	1.9		15.8	1.6

*Significant at $p \leq 0.05$ levels of probability

The mix of turnip/forage sorghum x sudangrass/sweet sorghum blend/forage pea/hybrid brassicas/oat/faba bean/forage pearl millet (Treatment 4) had a CP concentration of 150 g kg⁻¹, which more ($p \leq 0.05$) than all other treatments in the first harvest at Fargo-2019 (Fig 4.6A). Forage sorghum x sudangrass (Treatment 12) had the least CP ($p \leq 0.05$) during the first and second harvest at Fargo-2019 (Fig. 4.6A). The hybrid brassica/turnip mix (Treatment 2) had a CP of 157 g kg⁻¹ ($p \leq 0.05$) and was higher ($p \leq 0.05$) than of all treatments in the second harvest at Fargo-2019 (Fig. 4.6A). Treatment 3 and 12 had similar TDN, 697 g kg⁻¹ and 696 g kg⁻¹, in the first harvest and were the lowest ($p \leq 0.05$) of all treatments (Fig. 4.7A). Forage pearl millet (Treatment 10) had a TDN of 754 g kg⁻¹ in Fargo-2019 (Fig. 4.7A).

Forage pearl millet (Treatment 10) had a CP of 151 g kg⁻¹ for the first harvest in Fargo-2020 and was the highest ($p \leq 0.05$) among treatments (Fig. 4.6B). Forage sorghum x sudangrass

(Treatment 12) had a CP and TDN of 98 g kg⁻¹ and 653 g kg⁻¹, which was the lowest ($p \leq 0.05$) in the second harvest of Fargo-2020 (Fig. 4.6B, Fig. 4.7B). The forages in the second harvest had a reduction in CP in all treatments likely due to water stress that inhibit regrowth after the first harvest (Fig. 4.6B). Plants' capacity to absorb nitrogen decreases during drought circumstances, creating a decreased ion mobility in the soil produced by low soil moisture and restricted microbial activity around the plant root (Cregger et al., 2014; Gessler et al., 2017).

Annual ryegrass/chicory/plantain/red clover mix (Treatment 1) produced the highest CP (157 g kg⁻¹) and TDN (714 g kg⁻¹) in the first harvest at Hickson-2021 (Fig. 4.6C). In the second harvest, there was a significant ($p \leq 0.05$) increase in CP concentration in all treatments, excluding Treatment 1. Increase in nutritive value in the regrowth was likely due to an increase in leaf to stem ratio likely caused by the late rainfall in late August and September which promoted growth (Fig 4.1C). The brassica mixes of turnip/hybrid brassica had a CP of 172 g kg⁻¹, which was higher ($p \leq 0.05$) than the other treatments at Hickson-2021 in the second harvest (Fig. 4.6C). The brassica mix (Treatment 2) also produced the highest CP and TDN in the only harvest in the fall at Streeter-2021 (Fig 4.6D, Fig. 4.7D).

Total digestible nutrient is a commonly used metric for estimating the energy content of forages for grazing animals (Omokanye et al., 2019). Previous research (Bainard et al., 2020; Omokanye et al., 2019; Sanderson et al., 2018) found that annual forage mixes with diverse functional groups had higher nutritive value than monocrops. These functional groups included legumes' ability to fix atmospheric di-nitrogen, thus improving soil nutrients availability (Dahmardeh et al., 2010) and brassicas and grasses high capacity to scavenge and store nitrogen to prevent nutrient losses (Blanco-Canqui et al., 2015). However, in this research the CP and TDN values of mixes and monocrops varied across environments and among treatments. The

difference in the nutritive value between treatments varied largely due to the botanical composition of mixes (Bainard et al., 2020; Omokanye et al., 2019). In Fargo-2019 where the other species (brassicas and legumes) dominated the forage sorghum in the mix (Fig 4.5A), CP concentration significantly ($p \leq 0.05$) surpassed monocrops CP concentration (Fig. 4.7A). However, at Hickson-2021 (Fig 4.5C) there was no difference ($p > 0.05$) between the CP of mixes and monocrops as both mixes were predominantly forage sorghum (Fig. 4.7C).

The difference in environments due to rainfall and soil type at each location likely affected the nutritive value of forages. The lack of rainfall at Streeter-2021 slowed the growth rate and increased CP, as both mixes and monocrops were in the vegetative stage (before heading) at the time of harvest. The high CP value was due to the harvest period still in the vegetative stage and before heading). Crude protein in plant tissues usually decreases with plant maturity. The stage of plant maturity and its interaction with its environment (soil type, temperature, and rainfall) affects the chemical composition of forages (Nordheim-Viken et al., 2009).

The lack of rainfall at Streeter-2021 and during the second harvest at Fargo-2019 and Fargo-2020 was the likely cause of a decrease in TDN. Drought stress lowers nutrient uptake by the roots because of a lack of soil moisture, limiting the diffusion of nutrients from the soil to the roots and the activity of the nitrogenase enzyme (Hu et al., 2007). In addition, plants structurally respond to drought by increasing cell wall thickness in order to prevent water loss (Van der Weijde et al., 2017). The increase in cell wall thickness reduces fiber digestibility, which reduces TDN (Adesogan et al., 2014). All treatments surpassed the needed TDN for growing and finishing calves (650-700 g kg⁻¹), dry gestating cows (550-600 g kg⁻¹), and lactating cows (650 g kg⁻¹) (NASEM, 2016).

Forage brassicas were included in dual combinations of forage pearl millet/hybrid brassica (Treatment 5) and radish/forage sorghum x sudangrass (Treatment 6) to improve the forage nutritive value of the mixtures (Treatment 9 and 11). Neither radish nor hybrid brassica increased the CP and TDN in the mixes with forage sorghum x sudangrass at each harvest across locations, as there was no difference ($p > 0.05$) in CP and TDN between monocrops of forage pearl millet and forage sorghum x sudangrass and mixes (Fig 4.6D, Fig. 4.7D). This is not surprising given that the comparison of dual mixes and monocrops was the same as comparing monocrops against monocrops since the botanical makeup of the dual mixes was dominated by forage pearl millet and forage sorghum x sudangrass, respectively.

Grass/forage brassicas/legume mix of more than four species (Treatments 3 and 4) had CP in the range of 75 to 175 g kg⁻¹ (Fig 4.6). The turnip/forage sorghum x sudangrass/sweet sorghum blend/forage pea/hybrid brassicas/oat/faba bean/forage pearl millet mix (Treatment 4) outperformed the mix of hybrid brassica/oat/forage pea/forage sorghum x sudangrass/sweet sorghum blend/foxtail millet (Treatment 3) in CP and TDN concentration, especially in environments where the percentage of forage sorghum in the mix (Fig. 4.5) was less than the other species in the second harvest (Fargo-2019 and Fargo-2021). This might be related to the presence of more than one annual legume in Treatment 4 as opposed to Treatment 3. Because of their ability to fix atmospheric N₂ in symbiosis with *Rhizobia*, annual legumes in grass/legume mixes can boost the nutritive value of the mix (Finney and Kaye, 2016).

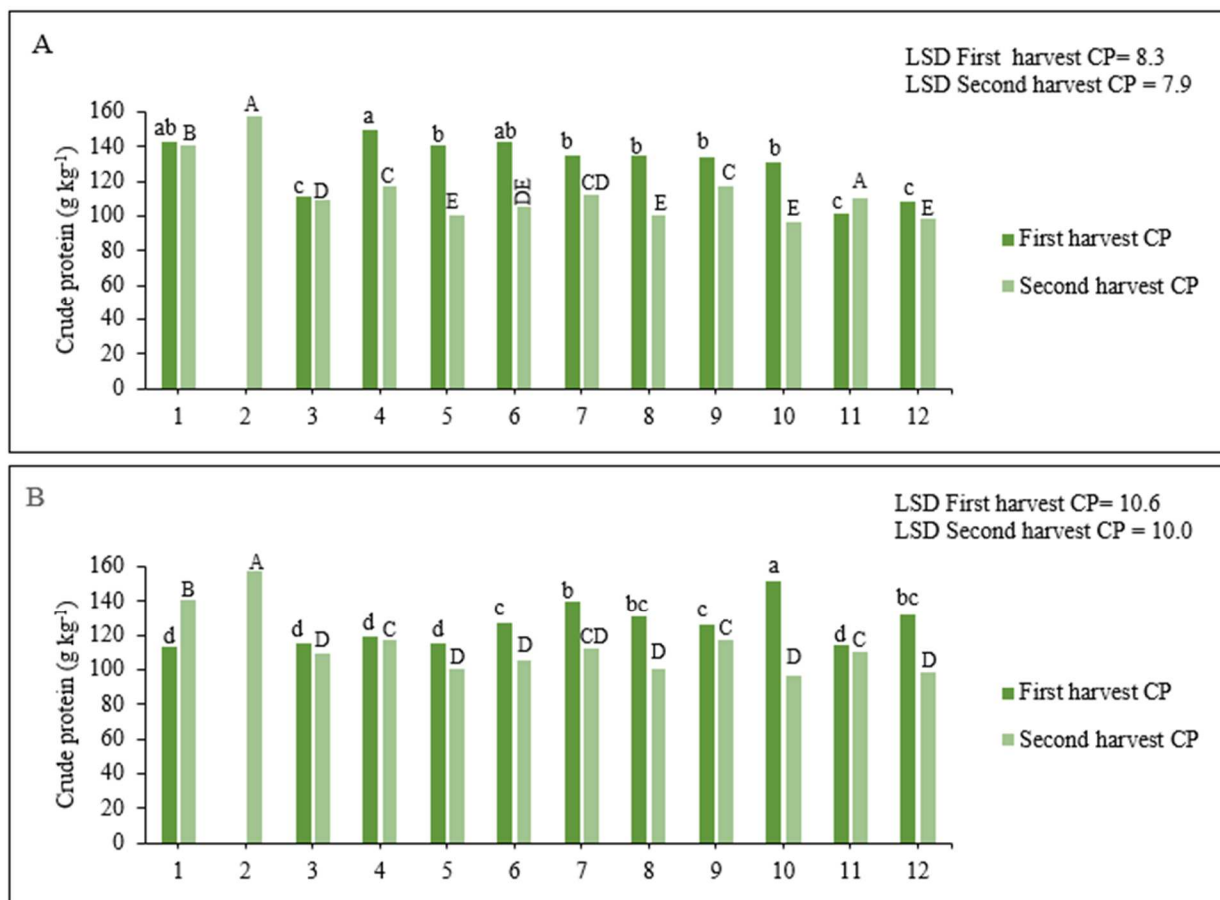


Figure 4.6. Mean crude protein (CP) concentration of forage mixes and monocrops harvested twice in the season in A) Fargo-2019, B) Fargo-2020, C) Hickson-2021, and D) Streeter-2021. Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In the same figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

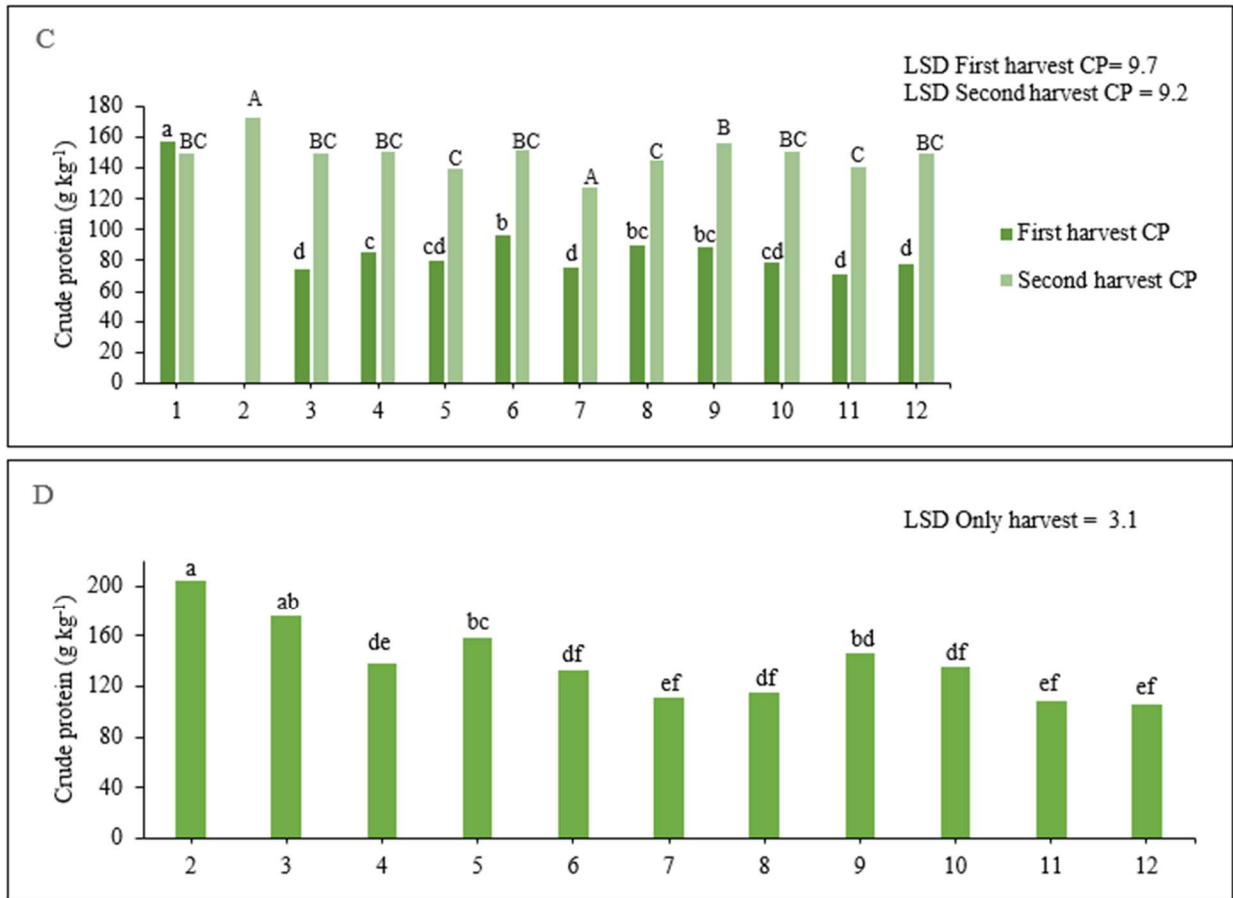


Figure 4.6. Mean crude protein (CP) concentration of forage mixes and monocrops harvested twice in the season in A) Fargo-2019, B) Fargo-2020, C) Hickson-2021, and D) Streeter-2021. (continued). Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/ sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In the same figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

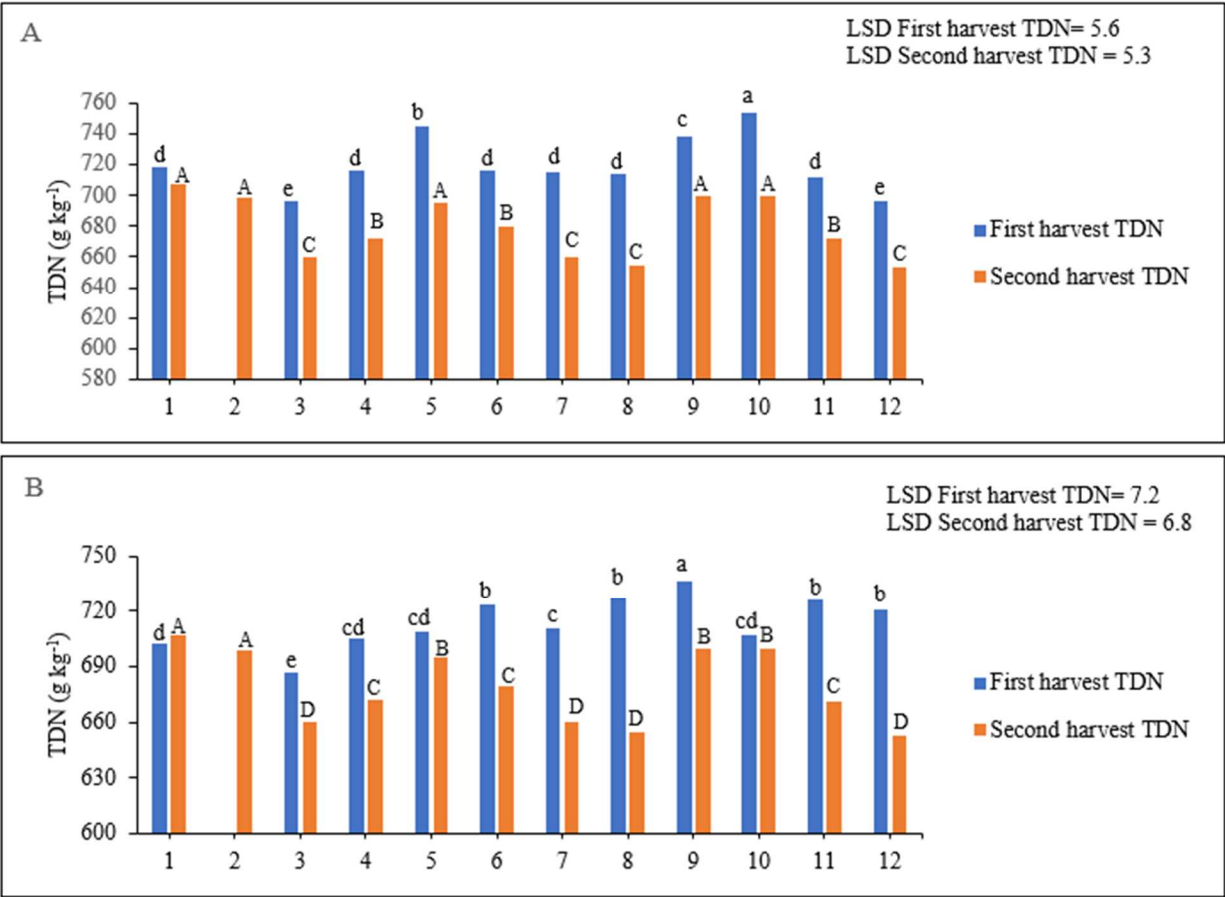


Figure 4.7. Total digestible nutrients (TDN) of annual forage mixes and monocrops harvested twice in the season in A) Fargo-2019, B) Fargo-2020; C) Hickson-2021, and D) Streeter-2021. Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In the same figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

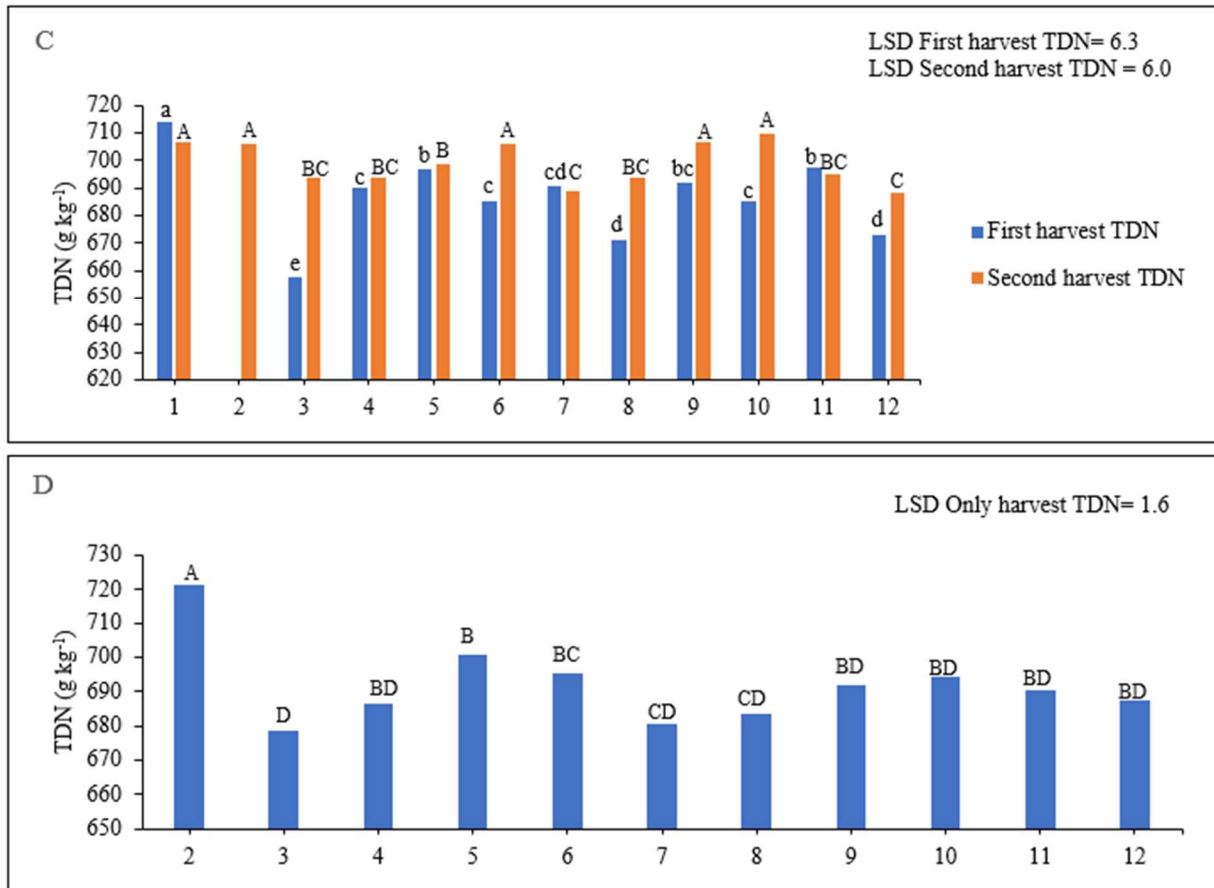


Figure 4.7. Total digestible nutrients (TDN) of annual forage mixes and monocrops harvested twice in the season in A) Fargo-2019, B) Fargo-2020; C) Hickson-2021, and D) Streeter-2021. (continued) Forage mixes (1 – 7): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica and turnip; 3) hybrid brassica, oat, forage pea, and forage sorghum x sudangrass/sweet sorghum blend and foxtail millet; 4) turnip, forage sorghum x sudangrass/ sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean and forage pearl millet; 5) forage pearl millet and hybrid brassica; 6) forage sorghum x sudangrass and radish; 7) oat, phacelia, forage pea, faba bean, and forage sorghum. Monocrops (8 -12): 8) forage sorghum x sudangrass/sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; and 12) forage sorghum x sudangrass. In the same figure, different letters between columns of same color indicate significant difference ($p \leq 0.05$).

4.8. Seed Cost Analysis

Table 4.10 illustrates the seed cost per dry matter yield produced by annual forage mixes and monocrops at different environments. Annual ryegrass/chicory/plantain/red clover mix (Treatment 1) was the most expensive mix compared with all other mixes and monocultures in all locations excluding Streeter-2021, in which this mix did not establish due to limited rainfall after planting (Table 4.10). Seed cost per Mg of biomass dry matter yield of Treatments 3-12 were similar in Fargo-2019 and Hickson-2021. Forage sorghum x sudangrass monocrop (Treatment 12) was the least expensive treatment in Fargo-2020 (Table 4.10).

The yield and seed cost per Mg of biomass yield produced have an inverse relationship, thus the greater the biomass yield produced, the lower the seed cost per Mg of biomass produced. Combinations incorporating winter rye (*Secale cereale* L.) and/or brassicas in three-way mixes were more expensive per Mg of dry matter yield than oat and clover mixes (Farney et al., 2018). In our study, mixes containing oat had forage brassicas included, except for Treatment 7. The only mix that had red clover and annual ryegrass was Treatment 1. The blend of annual ryegrass/chicory/plantain/red clover was the most expensive mix and also the least producing with all harvests summed up, making it the mix with the highest seed cost per Mg of dry matter yield (Table 4.11).

Mixes combining oat and brassicas produced similar yields (Treatments 3 and 4), but the turnip/forage sorghum-x-sudangrass/sweetsorghum blend/forage-pea/hybrid-brassicas/oat/faba bean/forage pearl millet mix (Treatment 4) was more expensive due to the number of species present and the cost of individual species in the mix (Table 4.11). The cost component of yearly mixes is heavily influenced by the price of seed and the amount of distinct species included in a

given mix, with smaller seeds having a lower recommended seeding rate, thus a lower total seed cost (Adjesiwor et al., 2017).

As in this study, the additional expense of adding species to the mixes was not compensated by greater biomass yield and or nutritive value as reported by Aasen et al. (2004). With the exception of the dual mix of radish and forage sorghum x sudangrass (Treatment 6), which was the lowest seed cost per Mg of biomass produced (Table 4.11), monocrops (Treatments 8-12) had lower seed costs per Mg of biomass produced than mixes.

The sensitivity study revealed the likely results of seed cost per Mg of biomass yield produced if seed price and yield increased or decreased, or if price just increased and biomass yield alone decreased (Table 4.11). Although not the most productive in terms of biomass yield, if prices rose by 10%, the radish/forage sorghum x sudangrass mixture (Treatment 7) was the most cost-effective annual forage, with a price range of \$2.4 Mg⁻¹ to \$2.9 Mg⁻¹ (Table 4.11). Forage sorghum x sudangrass/sweet sorghum blend monocrop (\$3.2 Mg⁻¹ - \$3.9 Mg⁻¹) should also be considered since it was the highest biomass producing treatment with an output of 12.4 Mg ha⁻¹.

Sanderson et al (2006) reported a six-species mix to be economically more viable than a nine-species mix. When compared with the other combinations including more than three species (Treatments 1 and 4), the six-species mix of hybrid brassica/oat/forage pea/forage sorghum x sudangrass/sweet sorghum blend/foxtail millet (Treatment 3) may be a better alternative for mixes with more than three species, as the seed cost per dry matter yield was the lowest at a comparatively high biomass output. However, in this study, considering that the N fertilizer input at all locations was the same for monocrops and mixes, the seed cost per dry matter yield of forage sorghum x sudangrass/sweet sorghum blend monocrop (Treatment 8) was lower than

Treatment 3, and forage sorghum x sudangrass/sweet sorghum blend monocrop yielded more than Treatment 3 across all locations. Hence, making the forage sorghum x sudangrass/sweet sorghum blend a better alternative to the hybrid brassica/oat/forage-pea/forage sorghum x sudangrass/sweet sorghum blend/foxtail millet mix.

Table 4.10. Seed cost per Mg of biomass yield produced by annual forages and mixes in Fargo, Hickson, and Streeter in 2019, 2020, and 2021.

Treatment	Fargo 2019	Fargo 2020	Hickson 2021	Streeter 2021	Yield [†]
	-----Cost \$/Mg-----				Mg ha ⁻¹
1	28.9	16.6	29.2	.	4.8
3	5.4	5.3	3.4	13.4	8.4
4	7.9	9.4	6.3	11.0	8.8
5	6.3	6.5	4.2	9.7	7.6
6	3.6	2.8	2.4	3.8	9.5
7	5.8	6.7	4.1	17.2	7.5
8	5.9	2.9	2.9	6.8	12.4
9	6.9	4.1	3.9	6.9	10.8
10	7.8	3.5	4.2	7.6	10.8
11	6.7	2.9	3.2	6.3	11.9
12	4.7	2.4	2.6	4.6	11.8
LSD (0.05)	4.2	2.5	4.7	2.6	1.4

Treatment (1 – 12): 1) annual ryegrass, chicory, plantain, and red clover; 2) hybrid brassica, turnip; 3) hybrid brassica, oat, forage pea, forage sorghum x sudangrass x sweet sorghum blend, foxtail millet; 4) turnip, forage sorghum x sudangrass x sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, forage pearl millet; 5) forage pearl millet, hybrid brassica; 6) forage sorghum x sudangrass, radish 7) oat, phacelia, forage pea, faba bean, forage sorghum; 8) sorghum x sudangrass x sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; 12) forage sorghum x sudangrass.

† Biomass yield averaged across four environments for each treatment

Table 4.11. Sensitivity analysis of the seed cost per Mg of dry matter yield produced (SCDM) of annual forages averaged across environments.

Trt	Yield †	10% increase in yield	10% decrease in yield	Seed price	10% increase in seed price	10% decrease in seed price	Average SCDM	10% price increase only SCDM	10% yield increase only SCDM	10% yield decrease only SCDM
	-----Mg/ha-----				-----\$/ha-----			-----\$/Mg-----		
1	4.8	5.3	4.3	111.6	122.8	20.9	23.2	25.5	21.1	25.8
3	8.4	9.2	7.5	39.9	43.8	4.3	4.8	5.2	4.3	5.3
4	8.8	9.6	7.9	63.1	69.4	6.5	7.2	7.9	6.6	8.0
5	7.6	8.4	6.9	40.2	44.2	4.7	5.3	5.8	4.8	5.9
6	9.5	10.5	8.6	25.2	27.7	2.4	2.6	2.9	2.4	2.9
7	7.5	8.2	6.7	41.8	45.9	5.0	5.6	6.1	5.1	6.2
8	12.4	13.7	11.2	43.9	48.2	3.2	3.5	3.9	3.2	3.9
9	10.8	11.8	9.7	47.6	52.3	4.0	4.4	4.9	4.0	4.9
10	10.8	11.9	9.7	47.6	52.3	4.0	4.4	4.8	4.0	4.9
11	11.9	13.0	10.7	43.9	48.2	3.3	3.7	4.1	3.4	4.1
12	11.8	13.0	10.6	34.0	37.4	2.6	2.9	3.2	2.6	3.2

Trt: Treatment (1 – 12): 1) annual ryegrass, chicory, plantain and red clover; 3) hybrid brassica, oat, forage pea, forage sorghum x sudangrass x sweet sorghum blend x foxtail millet; 4) turnip, forage sorghum x sudangrass x sweet sorghum blend, forage pea, hybrid brassica, oat, faba bean, forage pearl millet; 5) forage pearl millet, hybrid brassica; 6) forage sorghum x sudangrass, radish 7) oat, phacelia, forage pea, faba bean, forage sorghum; 8) forage sorghum x sudangrass x sweet sorghum blend; 9) forage pearl millet; 10) forage pearl millet; 11) forage sorghum x sudangrass; 12) forage sorghum x sudangrass.

† Total biomass yield averaged across four environments.

5. CONCLUSIONS

The productivity and nutritive value of annual forage mixes were affected by the interplay of weather and location characteristics. Monocultures produced more biomass than mixes and rejecting our hypothesize. The three most productive mixes in comparison to the others were: 1) hybrid brassica/oat/forage pea/forage sorghum x sudangrass/sweet sorghum blend/foxtail millet, 2) turnip/forage sorghum x sudangrass/sweet sorghum blend/forage pea/hybrid brassicas/oat/faba bean/forage pea, and 3) forage sorghum x sudangrass/radish mixture.

The nutritional feed requirements of beef cattle production were fulfilled by all mixes and monocrops. Forage brassicas had little effect on the forage nutritive value of dual mixes containing forage sorghum. Although the annual ryegrass/chicory/plantain/red clover mix provided a high forage nutritive value for continuous grazing, the seed cost per Mg of dry matter biomass yield produced was the highest of all treatments. Forage sorghum x sudangrass/radish mixture was the most cost-effective mix. Less forage sorghum and pearl millet in a mix should be used in future studies as the seeding rate of forage sorghum and pearl millet mixture in this study was 2.2 kg ha^{-1} , yet they dominated all mixes in which they were present. Further investigation on the biomass yield and nutritive value of various alternative grass/brassica/legume mixes should be carried out to suggest more suitable and cost-effective annual forage mixes.

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APPENDIX

Table A1. Botanical proportion of individual species (% of total dry matter) in Fargo, ND, in 2019.

Treatment	First harvest	Second harvest
1	Annual ryegrass (80.6%), chicory (4.6%), plantain (14.0%), red clover (0.8%)	Annual ryegrass (85.7%), chicory (5.6%), plantain (7.3%), red clover (1.4%)
2	.	Hybrid brassica (45.9%), turnip (54.1%)
3	Hybrid brassica (0.5%), oat (11.6%), forage pea (6.7%), forage sorghum x sudangrass/sweet sorghum blend (11.9%), foxtail millet (69.3%)	Hybrid brassica (16.9%), oat (14.4%), forage pea (0.3%), forage sorghum x sudangrass/ sweet sorghum blend (64.6%), foxtail millet (3.8%)
4	Turnip (1.6%), forage sorghum x sudangrass/sweet sorghum blend (41.7%), forage pea (16.9%), hybrid brassica (0.6%), oat (20.9%), faba bean (1.4%), forage pearl millet (16.9%)	Turnip (7.5%), forage sorghum x sudangrass/sweet sorghum blend (44.5%), forage pea (0.1%), hybrid brassica (1.5%), oat (3.8%), faba bean (0.0%), forage pearl millet (42.6%)
5	Forage pearl millet (91.6%), hybrid brassica (8.4%)	Forage pearl millet (93.8%), hybrid brassica (6.2%)
6	Forage sorghumx sudangrass (94.3%), radish (5.7%)	Forage sorghum x sudangrass (78.6%), radish (21.4%)
7	Oat (46.4%), phacelia (7.4%), forage pea (6.5%), faba bean (1.6%), forage sorghum (38.1%)	Oat (17.9%), phacelia (0.7%), forage pea (2.0%), faba bean (0.2%), forage sorghum (79.2%)

Table A2. Botanical proportion of individual species (% of dry matter) in Fargo, ND, in 2020.

Treatment	First harvest	Second harvest
1	Annual ryegrass (0%), chicory (84.2%), plantain (11.3%), red clover (4.5%)	Annual ryegrass (19.6%), chicory (65.5%), plantain (14.9%), red clover (0%)
2	.	.
3	Hybrid brassica (24.9%), oat (14.8%), forage pea (8.9%), forage sorghum x sudangrass/sweet sorghum blend (27.6%), foxtail millet (23.8%)	Hybrid brassica (17%), oat (17.3%), forage pea (0%), forage sorghum x sudangrass/ sweet sorghum blend (65.4%), foxtail millet (0.3%)
4	Turnip (24.3%), forage sorghum x sudangrass/sweet sorghum blend (29.2%), forage pea (24.8%), hybrid brassica (15.9%), oat (2.7%), faba bean (0%), forage pearl millet (3.1%)	Turnip (20.2%), forage sorghum x sudangrass/sweet sorghum blend (33.5%), forage pea (3.3%), hybrid brassica (9.2%), oat (1.9%), faba bean (0.0%), forage pearl millet (31.9%)
5	Forage pearl millet (52.9%), hybrid brassica (47.1%)	Forage pearl millet (83.8%), hybrid brassica (16.2%)
6	Forage sorghum x sudangrass (79.3%), radish (20.7%)	Forage sorghum x sudangrass (62.1%), radish (37.8%)
7	Oat (6.0%), phacelia (48.4%), forage pea (10.6%), faba bean (0.6%), forage sorghum (35%)	Oat (13.7%), phacelia (0.7%), forage pea (1.2%), faba bean (0%), forage sorghum (84.4%)

Table A3. Botanical proportion of individual species (% of total dry matter) in Hickson, ND, in 2021

Treatment	First harvest	Second harvest
1	Annual ryegrass (31.8%), chicory (61.0%), plantain (7.2%), red clover (0%)	Annual ryegrass (36.4%), chicory (60.9%), plantain (2.7%), red clover (0%)
2	.	.
3	Hybrid brassica (0%), oat (4.9%), forage pea (0%), forage sorghum x sudangrass/sweet sorghum blend (47.7%), foxtail millet (47.4%)	Hybrid brassica (0%), oat (0%), forage pea (0%), forage sorghum x sudangrass/sweet sorghum blend (100%), foxtail millet (0%)
4	Turnip (1.9%), forage sorghum x sudangrass/sweet sorghum blend (64%), forage pea (6.4%), hybrid brassica (0.7%), oat (22.8%), faba bean (0%), forage pearl millet (4.2%)	Turnip (0.9%), forage sorghum x sudangrass/sweet sorghum blend (70.1%), forage pea (0%), hybrid brassica (0%), oat (0%), faba bean (0%), forage pearl millet (29%)
5	Forage pearl millet (99.8%), hybrid brassica (0.2%)	Forage pearl millet (100%), hybrid brassica (0%)
6	Forage sorghum x sudangrass (98.9%), radish (1.1%)	Forage sorghum x sudangrass (96.2%), radish (3.8%)
7	Oat (6.0%), phacelia (48.4%), forage pea (10.6%), faba bean (0.6%), forage sorghum (35%)	Oat (2.9%), phacelia (0%), forage pea (0%), faba bean (0%), forage sorghum (97.1%)

Table A4. Number of seeds planted per plot across all locations

Species	1000- seeds weight	Pure live seed weight	Number of seeds per m ²	Number of seeds planted per plot
	-----g-----			
Annual ryegrass	4.8	20.8	24	4333
Chicory	1.2	3.0	14	2500
Plantain	2.7	4.5	9	1667
Red clover	2.6	3.9	8	1500
Hybrid brassica	4.9	2.2, 1.1‡	3, 1	449, 225
Turnip	1.8	1.1, 2.2	3, 7	611, 1222
Radish	8.1	2.3	2	284
Oat	21.6	5.3, 2.1	2, 1	245, 97
Forage pea	172.5	5.3	1	31
Faba bean	219.6	2.1, 5.3	1, 1	10, 24
Forage sorghum x sudangrass and sweet sorghum blend	29.9	12.3, 2.5	3, 1	537, 109
Forage pearl millet	8.0	12.3	9	1538
Forage pearl millet	8.0	12.3	9	1538
Forage sorghum x sudangrass	31.3	12.3, 2.5	3, 1	393, 80
Forage sorghum	35.3	12.3, 3.7	3, 1	348, 105
Phacelia	2.0	1.2	3	600
Foxtail millet	2.6	3.5	8	1346