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Comparison of alfalfa mixed with tall fescue and bermudagrass on forage accumulation, botanical composition, and nutritive value

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Abstract

In order to utilize alfalfa (*Medicago sativa* L.), alone or in mixtures with grasses, defoliation management practices must be evaluated to assess their performance. The objective was to determine the forage accumulation (FA) and nutritive value of alfalfa grown as a monoculture (ALF) and in mixtures with tall fescue [*Lolium arundinaceum* (Schreb.) Darbyish] (ATF) or bermudagrass [*Cynodon dactylon* (L.) Pers] (ABG) subjected to four harvest intervals (clipped every 21, 28, 35, and 42 d). The study was conducted in Crossville, TN, and Charleston, OH, during the 2016 and 2017 growing seasons, and in Salisbury, NC, during the 2017 and 2018 growing seasons. Harvest intervals of 35 d or greater showed optimal FA, with greatest productivity in spring. In summer, the plot productivity of ATF was not different from ABG. The ATF mixture was superior to ABG in FA for the entire season. Although tall fescue can be very competitive with alfalfa in mixtures, it results in greater FA while reducing weed competition. Botanical composition indicated greater weed infestation in ALF than in mixtures. Growing alfalfa–grass mixtures can increase sward crude protein compared with grass monocultures (average of 128 g kg^{-1} for ATF and 161 g kg^{-1} for ABG). We conclude that harvest intervals of 35 d or longer should be adopted to provide greater FA, and the ALF and ATF treatments resulted in superior FA compared with ABG in the southern United States.

1 | INTRODUCTION

Alfalfa (*Medicago sativa* L.) requires appropriate management and site selection to maximize productivity (such as high pH and deep soils) and the longevity of the stand (Ball et al., 2007), and maintain its nutritive value. Alfalfa can also be beneficial when grown as a mixture with perennial grasses (Aponte et al., 2019). Grass–legume mixtures can contribute to a more uniform distribution of forage production throughout the season through a complementary effect among species (Aponte et al., 2019). Considering that cool- and warm-season grasses thrive at different times during the growing season, it is possible to use alfalfa mixed with grasses to increase the length of the grazing season.

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbyish] is a widely adopted cool-season grass in the United States (Hoveland, 1993) because of its easy establishment and persistence over a wide range of conditions. In a study conducted by Lauriault et al. (2003), the authors concluded that an alfalfa–tall fescue mixture can maintain forage accumulation (FA) for longer periods than grass monocultures and decrease the use

Abbreviations: ABG, Alfalfa–bermudagrass mixture; ALF, Alfalfa monoculture; ATF, Alfalfa–tall fescue mixture; CP, Crude protein; FA, Forage accumulation; NDF, Neutral detergent fiber of synthetic N fertilizers, especially during spring, when tall fescue is actively growing (Mooso & Wedin, 1990). However, during the summer slump, which negatively affects tall fescue growth, bermudagrass [*Cynodon dactylon* (L.) Pers] can be an alternative for maintaining grass growth (Nelson & Burns, 2006).

Brown and Byrd (1990) compared the FA of alfalfa and bermudagrass in a monoculture or in mixtures, without N fertilization or receiving three levels of N fertilization. Mixing alfalfa with bermudagrass provided similar FA to alfalfa in a monoculture and bermudagrass monocultures (receiving 200 kg N ha^{-1}). Although several benefits can be obtained from legume–grass mixtures, shifts in botanical composition occur with environmental changes that can affect the forage nutritive value of the mixtures (Belesky et al., 2002). There is a positive relationship between forage legume proportion and crude protein (CP) concentration caused by N fixation by the legumes, whereas the grass proportion is correlated with amylase-treated neutral detergent fiber (NDF) concentration (Amiri & Shariff, 2012).

Forage nutritive value is closely related to harvest interval (Moore & Jung, 2001) and the stage of physiological maturity of plants. Longer harvest intervals will result in increased forage accumulation, but since there will be an advancement in plant maturity, forage nutritive value is usually reduced. This advanced maturity will result in loss of leaves and thickening of stems, reducing CP concentration and digestibility (Brink et al., 2010; Buxton et al., 1985; Henderson & Robinson, 1982; Nave, Sulc, Barker, & St-Pierre, 2014).

Adjusting the harvest interval in legume–grass mixtures is an important management decision for balancing the goals of FA (Arnold et al., 2019) and nutritive value, depending on the requirements of the livestock being fed. The objectives of this study were to evaluate the effects of four harvest intervals on the FA, botanical composition, and nutritive value of alfalfa in a monoculture and in mixtures with bermudagrass and tall fescue. Since FA is generally negatively correlated with nutritive value, it was hypothesized that adjustments in harvest intervals according to each species combination could be used as a tool for optimizing both FA and nutritive value for a specific class of livestock being fed the forage.

Core Ideas

- Alfalfa alone does not control the weed population as effectively as in tall fescue mixtures.
- Harvest intervals above 35 d allow better forage accumulation and nutritive value.
- Alfalfa mixed in tall fescue has greater accumulation and nutritive value than in bermudagrass.

2 | MATERIAL AND METHODS

2.1 | Treatments and experimental design

The study was conducted at three research stations across the United States. Two stations were located in the upper South (Tennessee and North Carolina) and one in the eastern Midwest (Ohio). The experimental design at the three locations was a randomized complete block with a split-plot arrangement of treatments. The whole-plot factor treatments were three forages: an alfalfa monoculture (ALF), an alfalfa–tall fescue mixture (ATF), and an alfalfa–bermudagrass mixture (ABG). The subplot factor treatments were four harvest intervals: clipped every 21, 28, 35, and 42 d. The Tennessee location had 48 experimental units (three forages × four harvest intervals × four replications). At the Ohio location, one replication was lost to herbicide drift in early August in 2016, resulting in 36 experimental units (three forages × four harvest intervals × three replications). The North Carolina location had only two forage treatments as whole-plot factors (ALF and ATF) and thus had 32 experimental units (two forages × four harvest intervals × four replicates). The experimental unit size across locations was 3 by 6 m. Specific dates and the number of defoliation events per location are presented in Table 1; cultivars and seeding rates are presented in Table 2.

2.2 | Site description, establishment, and management

At Crossville, TN (36˚01′N, 85˚1′W), the soil of the area is classified as a well-drained Lily series (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) soil complex with 2 to 6% slopes; at South Charleston, OH (39.86˚N, 83.67˚W), the soil type is a tile-drained Strawn (fine-loamy, mixed, active, mesic Typic Hapludalfs)–Crosby (fine, mixed, active, mesic Aeric Epiaqualfs) soil complex with 2 to 6% slopes; and at Salisbury, NC (35˚41′N, 80˚37′W), the soil of the area is classified as a well-drained Pacolet series (fine, kaolinitic, thermic Typic Kanhapludults) soil complex with 8 to 15% slopes. Precipitation and air temperature for each location are presented (Figure 1, Figure 2, Figure 3). Because drought conditions experienced in Tennessee in 2016, the plots were irrigated with 25 mm during the 2-d period of 23 and 24 Sept. 2016 via a gun on reel system. Soil amendments were applied according to each state's soil recommendations for alfalfa production on the basis of the initial soil test results (Table 2). The tall fescue cultivar used in Ohio was different from that in Tennessee and North Carolina, but according to tall fescue variety trial studies (NMSU+, 2020), 'Martin2' and 'Texoma Max QII'

TABLE 1 Harvest intervals implemented in Tennessee and Ohio during the 2016 and 2017 growing seasons, and the 2017 and 2018 growing season in North Carolina

Location	Harvest interval 21 d	28 d	35 d	42 d	21 d	28 d	35 d	42 d
	2016				2017			
Crossville, TN	3 May	10 May	17 May	24 May	2 May	9 May	16 May	23 May
	24 May	7 June	21 June	8 July	23 May	6 June	20 June	5 July
	14 June	8 July	26 July	16 Aug.	13 June	5 July	25 July	15 Aug.
	8 July	3 Aug.	30 Aug.	–	5 July	1 Aug.	29 Aug.	–
	26 July	30 Aug.	–	–	25 July	29 Aug.	–	–
	16 Aug.	–	–	–	15 Aug.	–	–	–
	–	–	–	–	5 Sept.	–	–	–
Charleston, OH	12 May	19 May	25 May	2 June	8 May	16 May	23 May	30 May
	2 June	16 June	30 June	14 July	30 May	13 June	27 June	11 July
	22 June	14 July	4 Aug.	24 Aug.	20 June	11 July	1 Aug.	22 Aug.
	14 July	11 Aug.	8 Sept.	–	11 July	8 Aug.	5 Sept.	–
	4 Aug.	8 Sept.	–	–	1 Aug.	5 Sept.	–	–
	24 Aug.	–	–	–	22 Aug.	–	–	–
Salisbury, NC	3 May	3 May	3 May	3 May	14 Apr.	14 Apr.	14 Apr.	14 Apr.
	26 May	31 May	9 June	14 June	11 May	11 May	21 May	24 May
	14 June	28 June	13 July	27 July	24 May	8 June	22 June	6 July
	6 July	27 July	15-Aug	19 Sept.	6 July	6 July	27 July	15 Aug.
	27 July	24 Aug.	19 Sept.	–	27 July	7 Aug.	28 Aug.	28 Sept.
	15 Aug.	19 Sept.	31 Oct.	–	15 Aug.	28 Aug.	5 Oct.	8 Nov.
	8 Sept.	25 Oct.	–	–	4 Sept.	28 Sept.	8 Nov.	–
	17 Oct.	–	–	–	28 Sept.	29 Oct.	–	–
	–	–	–	–	18 Oct.	–	–	–
	–	–	–	–	8 Nov.	–	–	–

TABLE 2 Forages seeding rates, planting dates, and initial soil nutrient levels at each location

State	Species	Cultivar	Alfalfa seeding	Planting date	Soil pH	P	K	Ca	Mg
						kg ha^{-1}			
Crossville, TN	Alfalfa	Ameristand 403T Plus	17 kg ha^{-1}	16 July 2015	5.5	34	110	662	28
	Tall fescue	Texoma Max Q II	11 kg ha^{-1}	4 Sept. 2015					
	Bermudagrass	Vaughn #1[a]	0.83 m^3 ha^{-1}	16 July 2015					
Charleston, OH	Alfalfa	Ameristand 403T Plus	26 kg ha^{-1}	10 Aug. 2015	6.4	60	154	2,035	404
	Tall fescue	Martin 2	13 kg ha^{-1}	1 May 2015					
	Bermudagrass	Wrangler	11 kg ha^{-1}	12 May 2015					
Salisbury, NC	Alfalfa	Ameristand 403T Plus	22 kg ha^{-1}	5 Oct. 2016	5.5	181	123	956	279
	Tall fescue	Texoma Max Q II	11 kg ha^{-1}	5 Oct. 2016					

[a]Vaughn (1994).

have similar forage responses in total dry matter yield, CP, and energy.

In Tennessee, the seedbed was conventionally tilled and bermudagrass was planted via vegetative propagation with small bales of bermudagrass and fertilized with 67 kg N ha^{-1} (34–0–0 N–P–K) on 16 July 2015. The ATF mixture was planted with a 25.4-cm row spacing no-till drill (Great Plains). On 7 Oct. 2015, N fertilizer was applied at a rate of 67 kg ha^{-1} to the ATF mixture to aid the establishment of the tall fescue component. On 26 Oct. 2015, all plots were treated with lime

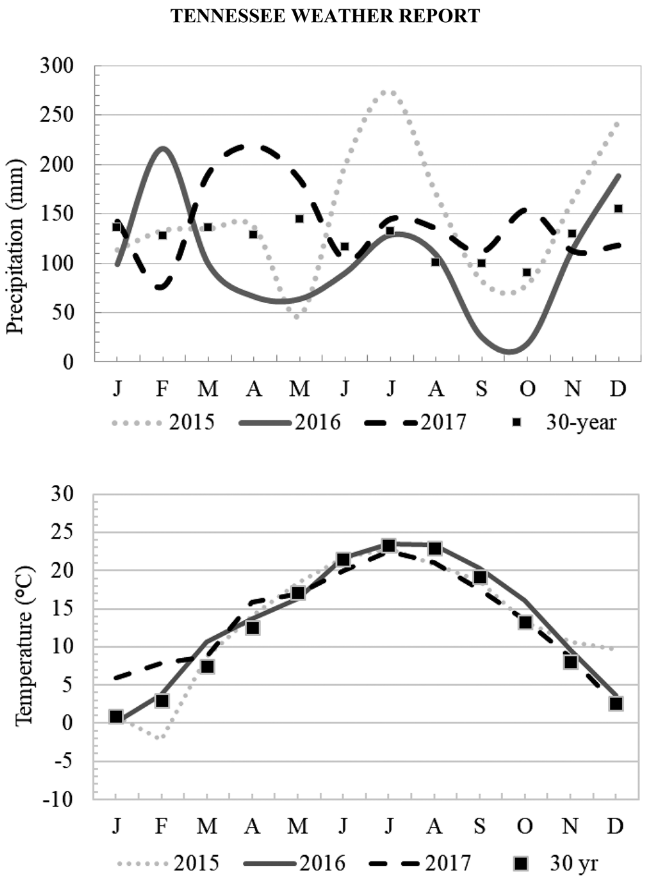

FIGURE 1 Temperature and precipitation for Crossville, TN, from 2015 to 2017, including the 30-yr average

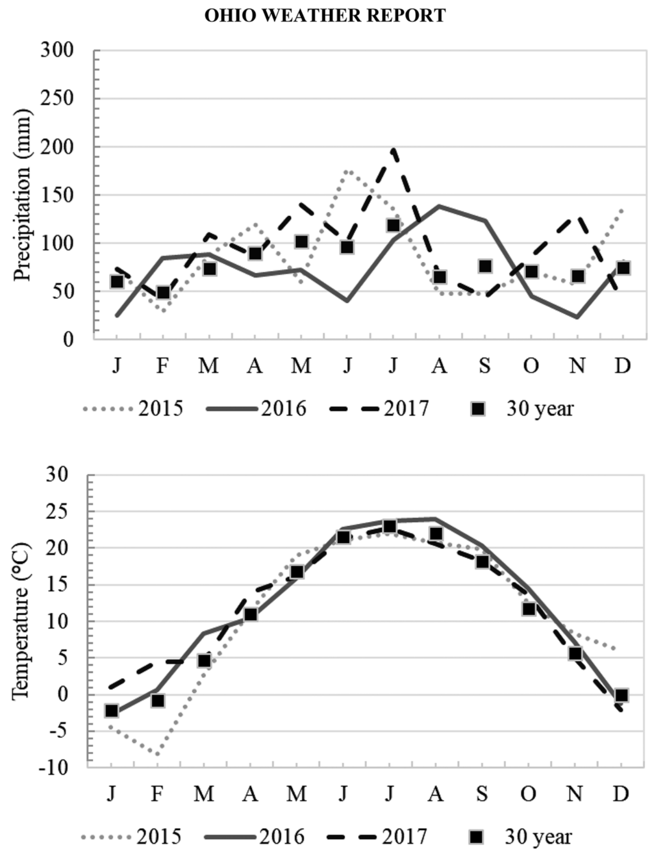

FIGURE 2 Temperature and precipitation for Charleston, OH, from 2015 to 2017, including the 30-yr average

at a rate of 6.1 Mg ha^{-1}. On 2 Feb. 2016 and 24 Feb. 2017, boron was applied to all plots at a rate of 2 kg ha^{-1}. Because of the low density of alfalfa in the ABG mixture, the alfalfa component of the mixture was replanted on 8 Mar. 2016 at a rate of 17 kg ha^{-1} with a small tube drill (Hege Company). On 13 Sept. 2016 and 19 Sept. 2017, all plots were clipped to a 10-cm stubble height to prepare for the growing season the following year.

In Ohio, tall fescue was planted on 1 May 2015. No fertilizer was needed according to Ohio State University Extension recommendations (Sulc et al., 2017). Tall fescue plots were clipped to an 8-cm stubble height on 11 June 2015, and the tall fescue and bermudagrass plots were clipped again on 10 Aug. 2015 just before alfalfa was no-till drilled across the entire experimental area, including the A treatment plots that had been maintained weed-free with glyphosate applications up to that point. The tall fescue and bermudagrass plots were clipped again on 2 Sept. 2015 at a clipping height just above the developing alfalfa seedlings. Herbicide and insecticide use in Tennessee and Ohio can be found in Table 3.

In North Carolina, the seedbed preparation process started on 22 Sept. 2016 by using turbo-till equipment (Titan Attachments) and incorporating lime at a rate of 4,480 kg ha^{-1}. One day before planting, the plots were turbo-tilled again, and the tall fescue and ATF plots were planted on 5 Oct. 2016 with a John Deere no-till drill (Model 1560; John Deere) in 16 rows 3 m wide at a 19-cm row spacing, and a cultipacker was run on top of the planted plots to ensure adequate soil–seed contact after planting. Irrigation was applied (31 mm) on 16 Nov. 2016 with a sprinkler system. Potassium was applied on 30 Mar. 2017 at a rate of 56 kg ha^{-1}. Phosphorus and potassium were applied on 5 Oct. 2017 at rates of 12 and 23 kg ha^{-1}, respectively. Boron was applied on 6 Oct. 2017 and 15 Aug. 2018 at 2.2 kg ha^{-1} in liquid form (10% Borosol, Loveland Products Inc.).

3 | RESPONSE VARIABLES

3.1 | Botanical composition

A feasibility calibration study was developed to determine the botanical composition following the guidelines and procedures outlined in Williams et al. (2017) and shown in Table 4. Re-prediction of the final percentages reported was then performed, with predictions fitting the allowable H statistics of less than 3.0 (Murray & Cowe, 2004; Shenk &

TABLE 3 Herbicide and insecticide usage in Tennessee and Ohio

State	Date	Herbicide	Rate	Species controlled
			a.i. kg ha^{-1}	
Crossville, TN	25 May 2016	2,4-DB 200 (a.i.: dimethlyamine salt of 4 (2,4 dichlorophenoxy) butyric acid; Agrisolutions, WinField)	1.1	Broadleaf weeds in alfalfa–bermudagrass plots
	6 Apr. 2017	Chlorpyrifos (DowAgrosciences)	0.47	Alfalfa weevil [*Hypera postica* (Gyllenhal)]
	9 May 2017	Chlorpyrifos (DowAgrosciences)	0.47	Potato leafhopper [*Empoasca fabae* Harris (Hemiptera: Cicadellidae)]
Charleston, OH	29 Apr. 2016 and 28 Apr. 2017	Warrior (lambda-cyhalothrin) (Syngenta)	0.03	Alfalfa weevil (*H. postica*)
	9 June, 12 July, 5 Aug., and 18 Aug. 2016; 20 June and 26 July 2017	Warrior (lambda-cyhalothrin) (Syngenta)	0.028	Potato leafhopper (*E. fabae*)

TABLE 4 Botanical composition calibration statistics and model information for the near-infrared reflectance spectroscopy

Statistic	Value
Sample set	n = 115 (five replications); ratios of 0–100% manually created from pure alfalfa and grass
Extreme set	n = 20; extremes of 0–10% and 90–100%
Expansion set	n = 98 (to incorporate real separation data)
Total spectra	n = 233
Math treatment	Partial least squares with seven factors selected and the outliers removed
Wavelengths used	1,100–2,400 nm
SD	0.14
Global distance	0.8
SE of cross-validation	1.51
Cross-validation	0.09
T-value	0.04

Westerhaus, 1991). The software used to develop this calibration model was UCAL (Unity Scientific).

Unstandardized spectra were collected for each ratio from 0 to 100% in 10% increments for each mixture. The initial spectra were used to make a calibration model to predict the botanical composition of field samples. The initial spectra collection included 115 total spectra, with five replications per category of grass to alfalfa percentages. Percentage reference values were associated with the scanned spectra. Expansion spectra were generated for the extremes of 0 to 10 and 90 to 100% with five duplicate scans per tier. Regression selections were made via the PLS function with a maximum factor of 7 used for the final model. Outliers and rejections were removed with outer limits of 3.000. For Tennessee and North Carolina, further model expansion was performed with an additional set of 98 field samples manually separated for the actual botanical composition of both the ATF and AB mixtures.

3.2 | Nutritive value

The samples collected during harvest were ground to pass a 1-mm sieve in a Wiley Mill (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co.) and used to determine the nutritive value. Samples from each harvest were analyzed for CP, NDF, and lignin using near-infrared reflectance spectroscopy (Unity SpectraStar XL-R, Unity Scientific). Spectra for the forage nutritive value analyses were standardized to a near-infrared reflectance spectroscopy master instrument on which 2016–2017 forage compositional grass hay and legume hay prediction equations were developed by the Near-Infrared Reflectance Spectroscopy Forage and Feed Consortium, Berea, KY. The software used was the complete chemometric calibration software package UCal 3 (Unity Scientific). The global *H* statistical test compared each sample against the population structure of the prediction equation calibration set

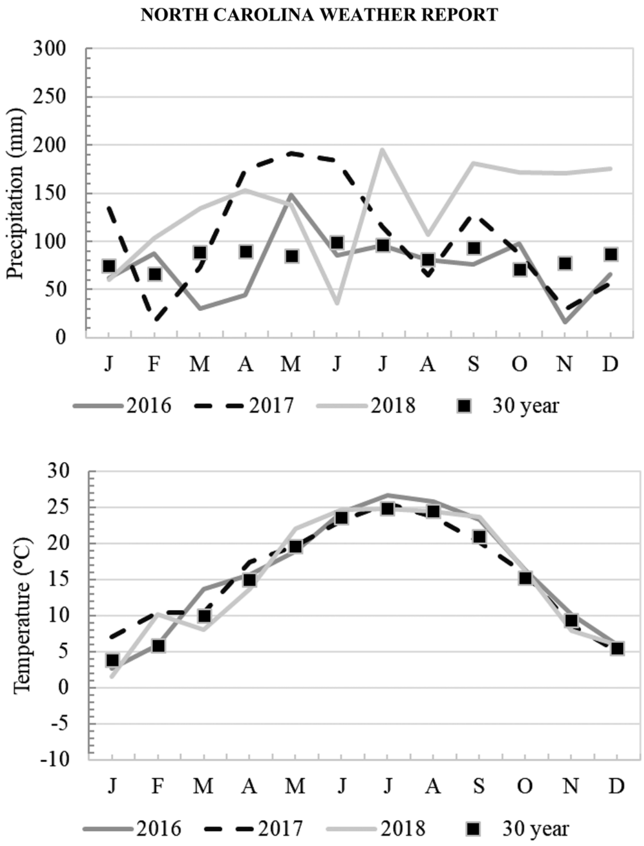

FIGURE 3 Temperature and precipitation for Salisbury, NC, from 2016 to 2018, including the 30-yr average

within the databases for valid results, where all forage samples fit the equation with H < 3.0 and are reported accordingly (Murray & Cowe, 2004).

3.3 | Forage accumulation

In Tennessee, to determine FA, a Carter forage harvester (Carter Manufacturing Company, Inc.) with a 0.94-m harvest swath width was used to collect the forage material of each experimental unit from the center of each plot. In Ohio, a 1.2- by 6.1-m center strip in each experimental plot was harvested with a sickle-bar forage harvester equipped with an electronic weighing system (Swift Machine and Welding). The border material of each plot was cleaned off after each harvest. In North Carolina, a 0.9- by 3-m area was harvested with a push-behind sickle bar mower (Jari Monarch) with a 0.9-m cutting width. The stubble height for all three states was 8 cm. In Tennessee and North Carolina, a subsample in the harvested material was used to determine dry matter concentration; in Ohio, forage from two to three randomly placed 0.1-m^2 quadrats was hand-clipped along the adjacent uncut areas of the plot. Once all the samples were collected, they were placed in a forced-air oven at 60 ˚C and dried to a constant weight (~72 h).

3.4 | Statistical analysis

Data were collected for two consecutive years: 2016 and 2017 in Tennessee and Ohio, and 2017 and 2018 in North Carolina. For the purpose of statistical inference, state, years, and seasons were analyzed individually. The FA was obtained by the sum of each harvest per experimental unit separated by year and by season; spring was considered to be May and June; summer was considered to be July, August, and September; and, for North Carolina, fall was October and November. Botanical composition, FA, and nutritive value variables were analyzed via a mixed-model ANOVA (PROC GLIMMIX, SAS 9.4, Cary, NC) to test the fixed effects of species, harvest interval, and their two-way interaction. The random effects included block, block × species, and block × species × harvest interval for botanical composition and nutritive values, and block and block × species for FA. If there was a significant harvest interval main effect, orthogonal polynomial contrasts were performed on FA. If significant interactions were found between species and harvest, the slicediff option of the LSmean option was used to assess the species effect when slicing by harvest interval. Least square means adjustments were performed via Tukey's honestly significant difference to control for Type 1 errors. Adjusted least square means and the SEMs are reported. The level of significance was determined at $\alpha = .05$.

4 | RESULTS AND DISCUSSION

4.1 | Weather

In 2016, dryer than normal weather occurred in both Tennessee and Ohio for a significant portion of the growing season (Figure 1, Figure 2). In Tennessee, the temperature was 0.2 ˚C above the 30-yr average and precipitation was 30% below the 30-yr average, whereas in Ohio, from April to July, the precipitation was also below the 30-yr average (Figure 2). In North Carolina, there were two abnormal weather conditions, with precipitation 50% above the 30-year average in 2017 and 44% above the 30-year average in 2018 (Figure 3).

4.2 | Botanical composition

In spring, Tennessee had a lower alfalfa concentration in ATF (both years: $P < .01$) and when the harvesting intervals were greater than 35 d in 2016 and greater than 28 d in 2017 ($P < .01$); the grass concentration was higher in ATF than in

ABG as expected during spring (Nave, Sulc, & Barker, 2013). Nevertheless, a quadratic contrast indicated that the 42-d harvest interval yielded the greatest grass proportions. There was an interaction observed in weed concentration in 2016 and 2017 (Table 5; $P < .01$), where a higher weed concentration was observed in the ALF plots and although longer harvest intervals have lower weed concentrations in ALF, this effect was not observed in the mixtures (Table 5). This was probably caused by the decreased available area and competition for light resulting from weed invasion. These results were also observed by Tracy et al. (2016),who showed that alfalfa–grass mixtures suppressed weeds more effectively. Similar results were observed in Ohio, with lower alfalfa concentrations in ATF across both years in the spring (Table 6), even with the interaction of species and harvest interval for alfalfa proportion in 2017, which resulted from small changes in the magnitude of differences among the treatments.

The higher alfalfa proportion during spring in ABG than in ATF was expected, since the optimal temperature for growing warm-season grasses (ranging from 25 to 40 ˚C) occurs mostly during summer (Bade et al., 1985; Mitich, 1989). Therefore, alfalfa is more competitive in spring, shading bermudagrass, resulting in a smaller proportion of grass. Alfalfa and tall fescue have similar photosynthetic pathways (Ball et al., 2007) and are therefore not as competitive with tall fescue during the cool season. According to Jung et al. (1996), alfalfa is not competitive with tall species such as tall fescue.

Although not in a clear pattern, the grass concentration in Ohio was generally higher with less frequent harvests ($P < .01$; Table 6), and the orthogonal polynomial contrasts showed a linear relationship with the greatest grass proportion at the 42 d harvest interval. In North Carolina, as expected, the ALF concentration was greater than that of ATF during the spring of 2017, which could also be observed in the species × harvest intervals of 2018 ($P < .01$; Table 7). Although harvest interval did not affect ALF, for ATF, a larger alfalfa proportion was observed for the 35 and 42 d harvest intervals (Table 7). Ventroni et al. (2010) concluded that with increased harvesting frequencies, the number of alfalfa shoots per m^3 increased, which could also be observed in our study.

With alfalfa and bermudagrass mixtures, it is important to consider establishing alfalfa into wide planting rows to allow bermudagrass to grow during the summer by reducing competition with alfalfa (Stringer et al., 1994). In the present study, alfalfa was not widely spaced when established into ABG (20 cm), which contributed to the higher alfalfa concentration in the mixture. However, even with reduced bermudagrass, ABG mixtures were still effective for weed control. In our study, higher weed proportions were observed for ALF as opposed to mixtures with grass.

During summer in Tennessee, only the effect of species was observed for alfalfa proportions in 2016, with ALF having a higher alfalfa concentration ($P < .01$, Table 5). However, in the summer of 2017 a species × harvest interval interaction was observed for alfalfa and grass concentrations ($P = .02$; Table 5). A larger alfalfa concentration was generally observed in ALF even though warmer summer temperatures are known to decrease the overall growth of alfalfa (Metochis & Orphanos, 1981). However, ABG and ATF differed as harvest intervals increased. Greater grass concentrations were observed in ATF with an increase in harvest intervals, but no clear differences in harvest intervals were observed. A species × harvest interval interaction was observed for weed concentration in 2016, but only the effect of species was observed in 2017 (Table 5). In general, higher weed concentrations were observed for ALF, especially at the 42 d interval ($P < .01$, Table 5).

Very similar results were observed during summer in Ohio, with a higher alfalfa proportion in ALF ($P < .01$, Table 6). In 2016, the Ohio grass proportion was higher for ATF than for ABG ($P < .01$); in 2017, ATF had higher grass proportion, but no clear pattern for differences in harvest interval could be seen (Table 6). An interaction was observed between species × harvest interval in the weed proportion in 2016 ($P = .04$, Table 6), although very few differences were observed among species. In general, there was a decrease in weed concentration for ABG and ATF as the harvest interval increased, but for ALF, only the 42 d frequency produced a smaller weed proportion, although it was not different from that of the 28 d frequency (Table 6). Additionally, only the effect of harvest interval occurred in 2017, with greater weed concentrations shown for more frequent harvest intervals. Orthogonal contrasts confirmed that weed proportions decreased linearly with longer harvest intervals.

Finally, during summer in North Carolina, very few differences were observed in the proportion of alfalfa, with only the effect of harvest interval seen during both years ($P < .01$, Table 7). In general, the 21-d harvest interval showed a smaller alfalfa proportion than the other intervals. The same pattern was observed during the fall of 2018, although no differences for alfalfa proportions were observed during the fall of 2017 (Table 7). These results were confirmed by a positive linear relationship for the alfalfa proportion, with increased alfalfa mass as the harvesting interval increased. Weed proportions showed no differences in the summer of 2017; however, a species × harvest interval interaction was observed during the summer of 2018 and during fall in both years. In general, ALF had a higher weed concentration than ATF, especially as the harvest interval decreased. Similar to observations in from Tennessee and Ohio, weed proportions decreased as the harvest interval increased ($P < .01$, Table 7).

During the second year after establishment, shorter harvest intervals produced an increase in weed proportion where these plants remained vegetative (Anower et al., 2017) because of a lack of flowering. Weeds were able to compete for sunlight

TABLE 5 Botanical composition (g kg^{-1}) of alfalfa (ALF) and mixed alfalfa–tall fescue (ATF) and alfalfa–bermudagrass (ABG) forages in Tennessee during spring and summer of 2016 and 2017

Harvest intervals		Alfalfa				Grass				Weeds			
		ALF	ABG	ATF	$\bar{X}_1$[a]	ALF	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
	d	g kg^{-1}											
Spring 2016	21	880	717	463	686A[b]	–	255	494	375B	120Ca	29Ab	43Ab	64
	28	909	702	633	748A	–	275	358	317B	91Ca	22Aab	10Ab	41
	35	700	555	283	513B	–	414	693	554A	300Ba	30Ab	24Ab	118
	42	512	552	46	370B	–	438	948	693A	488Aa	11Ab	7Ab	168
	$\bar{X}_2$	750a	652b	356c	–	–	345b	623a	–	250	23	21	–
	ANOVA												
	P-value (interaction)	.40[c]				.19				<.01			
	P-value (interval)	<.01				<.01				NA			
	P-value (species)	<.01				<.01				NA			
Harvest intervals		**Alfalfa**				**Grass**				**Weeds**			
		ALF	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
	d	g kg^{-1}											
Spring 2017	21	806	594	378	593A	–	383	601	492B	194C,a	24A,b	20A,b	798
	28	542	403	85	343B	–	558	886	722A	457A,a	39A,b	29A,b	175
	35	614	493	141	416B	–	479	834	656A	385AB,a	27A,b	25A,b	146
	42	726	515	137	460B	–	453	839	645AB	273BC,a	31A,b	24A,b	109
	$\bar{X}_2$	672a	501b	185c	–	–	468b	790a	–	328	30	24	–
	ANOVA												
	P-value (interaction)	.84				.61				<.01			
	P-value (interval)	<.01				<.01				NA			
	P-value (species)	<.01				<.01				NA			

(Continues)

TABLE 5 (Continued)

Harvest intervals		Alfalfa				Grass				Weeds			
		ALF	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
	d	g kg^{-1}											
Summer 2016	21	953	592	585	710	–	399	401	400	48Ba	9Aa	15Aa	24
	28	951	562	628	714	–	431	362	396	54Ba	7Ab	10Aab	24
	35	954	526	651	710	–	469	342	406	49Ba	5Ab	6Aab	20
	42	852	444	623	640	–	540	366	453	148Aa	17Ab	11Ab	60
	$\bar{X}_2$	928a	531c	622b	–	–	460	398	–	75	9	10	–
	ANOVA												
	P-value (interaction)	.65				.14				<.01			
	P-value (interval)	.07				.52				NA			
	P-value (species)	<.01				.08				NA			
Harvest intervals		**Alfalfa**				**Grass**				**Weeds**			
		ALF	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
	d	g kg^{-1}											
Summer 2017	21	654Ba	457Bb	318Ab	477	–	519Aa	647Ba	583	346	25	36	135
	28	761ABa	495ABb	221ABc	493	–	470ABb	721ABa	595	239	36	58	110
	35	867Aa	639ABb	69Bc	525	–	346ABb	900Aa	623	133	15	31	59
	42	803ABa	689Aa	250ABb	581	–	302Bb	737ABa	520	197	10	13	73
	$\bar{X}_2$	772	570	214	–	–	435	730	–	229a	21b	34b	–
	ANOVA												
	P-value (interaction)	.02				.02				.11			
	P-value (interval)	NA				NA				.07			
	P-value (species)	NA				NA				<.01			

[a] $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c] NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

TABLE 6 Botanical composition (g kg^{-1}) of alfalfa (ALF) and mixed alfalfa–tall fescue (ATF) and alfalfa–bermudagrass (ABG) forages in Ohio during the spring and summer of 2016 and 2017

Harvest interval		Alfalfa				Grass				Weeds			
		ALF	ABG	ATF	$\bar{X}_1$[a]	ALF	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
	d	g kg^{-1}											
Spring 2016	21	954	931	612	832	–	45	311	178B[b]	46	24	77	49a
	28	958	868	478	768	–	123	466	298A	42	9	57	36ab
	35	962	908	52	641	–	67	440	267AB	38	25	38	33b
	42	1000	912	370	761	–	88	627	370A	0	0	4	1c
	$\bar{X}_2$	963a	907a	526b	–	–	91b	464a	–	32ab	15b	44a	–
		ANOVA											
	P-value (interaction)	.24[c]				.17				.54			
	P-value (interval)	.40				<.01				<.01			
	P-value (species)	<.01				<.01				.02			
		Alfalfa				**Grass**				**Weeds**			
Harvest interval		**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
	d	g kg^{-1}											
Spring 2017	21	964Aa	898Ba	487Bb	783	–	88	504	296	36	14	9	20
	28	923Aa	937ABa	594Ab	818	–	62	406	234	77	2	0	26
	35	975Aa	996ABa	485Bb	819	–	4	515	260	25	0	0	8
	42	1,000Aa	1,000Aa	405Bb	802	–	0	595	298	0	0	0	0
	$\bar{X}_2$	961	945	503	–	–	64b	505a	–	35a	4b	3b	–
		ANOVA											
	P-value (interaction)	.04				.16				.06			
	P-value (interval)	NA				.21				.06			
	P-value (species)	NA				<.01				<.01			

(Continues)

TABLE 6 (Continued)

Harvest interval		Alfalfa ALF	Alfalfa ABG	Alfalfa ATF	Alfalfa $\bar{X}_1$	Grass ALF	Grass ABG	Grass ATF	Grass $\bar{X}_1$	Weeds ALF	Weeds ABG	Weeds ATF	Weeds $\bar{X}_1$
	d	g kg^{-1}											
Summer 2016	21	951	671	603	742B	–	238	326	282	49Ab	91Aa	70Aab	70
	28	956	777	656	796AB	–	197	304	251	44ABa	27Ba	40ABa	37
	35	936	775	701	804AB	–	192	284	238	64Aa	33Ba	15Ba	37
	42	1,000	865	724	863A	–	124	253	189	0Ba	12Ba	23Ba	12
	$\bar{X}_2$	960a	763b	663c	–	–	193b	296a	–	40	44	41	–
		ANOVA											
	P-value (interaction)	.46				.97				.04			
	P-value (interval)	<.01				.24				NA			
	P-value (species)	<.01				<.01				NA			
Harvest interval		**Alfalfa ALF**	**Alfalfa ABG**	**Alfalfa ATF**	**Alfalfa $\bar{X}_1$**	**Grass ALF**	**Grass ABG**	**Grass ATF**	**Grass $\bar{X}_1$**	**Weeds ALF**	**Weeds ABG**	**Weeds ATF**	**Weeds $\bar{X}_1$**
	d	g kg^{-1}											
Summer 2017	21	811Ba	722Bb	564Bc	699	–	239Ab	340Aa	289	189	39	102	110A
	28	914Aa	791Bb	537Bc	748	–	133BCb	391Aa	262	80	77	83	80A
	35	982Aa	882Aa	681Ab	848	–	118ABb	351Aa	234	9	0	9	6B
	42	988Aa	960Aa	675Ab	874	–	40Cb	353Aa	196	11	0	0	4B
	$\bar{X}_2$	911	822	582	–	–	143	361	–	89	35	57	–
		ANOVA											
	P-value (interaction)	.04				<.01				.08			
	P-value (interval)	NA				NA				<.01			
	P-value (species)	NA				NA				.09			

[a] $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

TABLE 7 Botanical composition (g kg^{-1}) of alfalfa (ALF) and mixed alfalfa–tall fescue (ATF) forages in North Carolina during the spring, summer, and fall of 2017 and 2018

Harvest interval	Alfalfa			Grass		Weeds			Alfalfa			Grass		Weeds		
	ALF	ATF	$\bar{X}_1$[a]	ALF	ATF	ALF	ATF	$\bar{X}_1$	ALF	ATF	$\bar{X}_1$	ALF	ATF	ALF	ATF	$\bar{X}_1$
	2017								2018							
d	g kg^{-1}															
Spring																
21	985	789	887	–	208	15Ba[b]	4A,a	9	923Aa	508Bb	716	–	472	77	20	48
28	979	822	901	–	170	21Ba	8Aa	14	955Aa	493Bb	724	–	475	45	32	38
35	989	836	912	–	161	11Ba	3Aa	7	956Aa	637Ab	797	–	342	44	21	32
42	921	795	858	–	196	79Aa	9Ab	44	926Aa	719Ab	822	–	281	74	0	37
$\bar{X}_2$	968a	810b	–	–	184	32	6		940	590	–	–	392	60a	18b	–
	ANOVA															
P-value (interaction)	.55[c]			–		<.01			<.01			–		.29		
P-value (interval)	.21			–		NA			NA			–		.77		
P-value (species)	<.01			–		NA			NA			–		<.01		
Harvest interval	**Alfalfa**			**Grass**		**Weeds**			**Alfalfa**			**Grass**		**Weeds**		
	ALF	**ATF**	$\bar{X}_1$	**A**	**ATF**	**ALF**	**ATF**	$\bar{X}_1$	**ALF**	**ATF**	$\bar{X}_1$	**ALF**	**ATF**	**ALF**	**ATF**	$\bar{X}_1$
	2017								**2018**							
d	g kg^{-1}															
Summer																
21	948	735	810B	–	224	52	40	46	387	419	402C	–	471	613Aa	111Ab	362
28	993	890	941A	–	95	7	14	11	608	491	549B	–	406	392Ba	103Ab	247
35	994	858	926A	–	127	6	15	10	755	659	707A	–	252	245Ca	89Ab	167
42	997	898	947A	–	88	4	15	9	769	539	654AB	–	352	232Ca	108Aa	170
$\bar{X}_2$	967	845	–	–	134	33	21	–	630	527	–	–	370	370	103	–
	ANOVA															
P-value (interaction)	.9			–		.76			.08			–		<.01		
P-value (interval)	<.01			–		.09			<.01			–		NA		
P-value (species)	.05			–		.89			.16			–		NA		

(Continues)

TABLE 7 (Continued)

Harvest interval	Alfalfa			Grass		Weeds			Alfalfa			Grass		Weeds		
	ALF	ATF	$\bar{X}_1$	A	ATF	ALF	ATF	$\bar{X}_1$	ALF	ATF	$\bar{X}_1$	ALF	ATF	ALF	ATF	$\bar{X}_1$
	2017								2018							
d	g kg^{-1}															
Fall																
21	998	768	883	–	124	3Ab	108Aa	55	193	389	291C	–	450	807Aa	161Ab	483
28	1,000	910	955	–	77	0Aa	13Ba	7	532	410	471B	–	432	468Ba	158Ab	313
35	1,000	977	989	–	15	0Aa	7Ba	4	769	555	600B	–	328	231Ca	116Ab	174
42	997	936	966	–	58	3Aa	6Ba	5	867	691	779A	–	221	133Da	88Aa	110
$\bar{X}_2$	998	897	–	–	69	2	34	–	559	512	–	–	357	409	131	–
	ANOVA															
P-value (interaction)	.06			–		<.01			.07			–		<.01		
P-value (interval)	.06			–		NA			<.01			–		NA		
P-value (species)	.08			–		NA			.36			–		NA		

[a] $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c] NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

TABLE 8 Total dry matter forage accumulation (kg ha^{-1}) during the spring and summer of 2016 and 2017 in Tennessee

	2016				2017			
Harvest interval	**ALF**[a]	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	kg ha^{-1}							
Spring								
21	5,810Ba[b]	650Bb	5,260Ca	3,910	4,260Ba	3,330Ca	3,370Ca	3,653
28	5,990Ba	320Bb	5,180Ca	3,830	5,690Aa	4,720Bb	5,000Bab	5,137
35	7,200Aa	1,730Ab	7,870Aa	5,600	6,270Aa	6,000Aa	6,670Aa	6,313
42	4,440Cb	1,020ABc	6,410Ba	3,960	4,020Bb	3,980BCb	5,520Ba	4,507
$\bar{X}_2$	5,860	930	6,180	–	5,060	4,500	5,140	–
	ANOVA							
P-value (interaction)	<.01[c]				<.01			
P-value (interval)	NA				NA			
P-value (species)	NA				NA			
	2016				**2017**			
Harvest interval	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	kg ha^{-1}							
Summer								
21	1,680	1,850	1,020	1,520C	1,340	1,540	1,340	1,410C
28	3,550	3,340	2,590	3,160AB	2,120	2,410	1,950	2,160B
35	3,020	3,320	2,810	3,050B	1,780	1,820	1,610	1,730C
42	3,350	3,800	3,580	3,580A	3,150	3,560	3,390	3,370A
$\bar{X}_2$	2,900	3,080	2,500	–	2,100	2,330	2,070	–
	ANOVA							
P-value (interaction)	.37				.91			
P-value (interval)	<.01				<.01			
P-value (species)	.11				.15			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.

[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).

[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

and resources when plots were harvested frequently, but this competition was reduced with longer regrowth intervals. This was especially important at the end of spring when annual summer weeds were starting to emerge (Ballare & Casal, 2000; Teasdale, 1993; Zimdahl, 2007) and compete effectively. Longer harvest intervals also enabled all forages to have enough time to reestablish their root carbohydrates, grow effectively, and compete for resources (Ball et al., 2007; Dhont et al., 2002; Li et al., 1996).

4.3 | Forage accumulation

In Tennessee, species × harvest interval interactions were observed during spring of both years (Table 8). For all three species during spring, FA was greatest for the 35-d interval, although it was not different from the 42-d interval for ABG in 2016 and from the 28 d interval for ALF in 2017 ($P < .01$, Table 8). In addition, during the spring of 2016, ATF showed greater FA than ABG and differed from ALF only at the 42 d interval, for which ATF had greater FA than ALF. A study conducted by Jung et al. (1996) with three different ryegrass cultivars mixed with alfalfa observed that taller cultivars were more competitive with alfalfa than shorter ryegrass cultivars. Although no comparisons with C_4 plants were made by Jung et al. (1996), Dhakal et al. (2020) found that warm-season native grass forages with alfalfa increased FA. In the spring of 2017 in Tennessee, ABG was lower in FA in only two comparisons (versus ALF for the 28 d interval and versus ATF for the 42-d interval; Table 8). In Ohio, the species × harvest interval interaction was only observed in the spring of 2016, and the effect of harvest interval was only observed only in the spring of 2017 ($P < .01$, Table 9). However, as opposed to the results found in Tennessee, ALF had greater FA than ATF, except at the 42-d interval in 2017; ATF generally did not differ from ABG (Table 9).

TABLE 9 Total dry matter forage accumulation (kg ha^{-1}) during the spring and summer of 2016 and 2017 in Ohio

	2016				2017			
Harvest interval	**ALF[a]**	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	kg ha^{-1}							
Spring								
21	9,010Aa[b]	3,670Bb	2,360Bb	5,010	6,110	5,980	7,150	6,410B
28	9,060Aa	5,460Ab	3,720Bc	6,080	6,020	5,680	6,690	6,130B
35	9,710Aa	5,940Ab	5,670Ab	7,110	8,660	6,510	9,720	8,290A
42	6,900Ba	4,550ABb	5,360Aab	5,600	4,660	4,740	5,940	5,110C
$\bar{X}_2$	8,670	4,900	4,280	–	6,360	5,720	7,380	–
	ANOVA							
P-value (interaction)	<.01[c]				.24			
P-value (interval)	NA				<.01			
P-value (species)	NA				.21			
Harvest interval	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	kg ha^{-1}							
Summer								
21	3,380	2,440	2,890	2,920C	3,700	2,770	2,570	3,010C
28	5,000	4,300	3,510	4,270B	5,040	4,450	4,080	4,520B
35	5,520	5,230	4,130	4,910AB	4,010	3,560	4,020	3,860BC
42	5,630	5,360	4,470	5,160A	7,030	6,960	6,570	6,850A
$\bar{X}_2$	4,890	4,280	3,770	–	4,940	4,430	4,310	–
	ANOVA							
P-value (interaction)	.88				.92			
P-value (interval)	<.01				<.01			
P-value (species)	.17				.78			

[a] ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in species effect ($P < .05$).
[c] NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

The pattern observed in Tennessee was also seen in Ohio regarding harvest intervals, with the 35 d interval consistently showing numerically greater FA during spring in both years, but was most consistently and statistically significant in 2017 (Table 9). During spring, very frequent harvest intervals (21 and 28 d) are detrimental for forage productivity and persistence (Quinby et al., 2020), whereas less frequent harvest intervals (42 d) lead to reduced FA, probably caused by the loss of leaves with advanced maturity at harvest time (Fuess & Tesar, 1968). In most instances, a harvest interval of 35-d appeared to be the most productive.

After reaching maturity, alfalfa plants dedicate resources into seed production instead of FA (Balde et al., 1993; Sosebee & Wiebe, 1973). Moreover, when harvested too frequently, plants are not able to regrow to their full potential and have less FA. Frequent harvests lead to lower leaf area index, which decreases the amount of solar radiation received by the plants and reduces the presence of photosynthetically active leaves, which affects the regrowth and vigor of the sward (Pedreira et al., 1999). In addition, longer harvest intervals allow roots to have more total nonstructural carbohydrates (Chaparro et al., 1996; Edmisten et al., 1988), which are important for shoot regrowth (Vonelec et al., 1996). It is important to maintain cool-season forages in vegetative stages during spring when there is rapid FA but, at the same time, prevent too long a harvest interval when the plant reaches maturity (Nave, Sulc, Barker, & St-Pierre, 2014).

In North Carolina, there was only the effect of harvest interval during the spring of both years (Table 10). In 2017, the 28 and 42 d intervals showed greater FA; however, in the spring of 2018, the 35 d interval also showed greater FA than the other harvest intervals. This was also verified by the orthogonal contrast, which showed a quadratic relationship, with the 35 d interval having greater FA and FA decreasing at 42 d.

During summer in Tennessee for both years, there was a harvest interval effect, with the 42-d interval showing greater

TABLE 10 Total dry matter forage accumulation (kg ha^{-1}) during spring and summer 2017 and 2018 in North Carolina

Harvest interval	2017 ALF[a]	2017 ATF	2017 $\bar{X}_1$	2018 ALF	2018 ATF	2018 $\bar{X}_1$
d	kg ha^{-1}					
Spring						
21	5,230[b]	5,380	5,310B	3,650	3,950	3,800C
28	6,510	7,240	6,880A	4,690	5,380	5,040B
35	3,840	5,330	4,590B	5,550	6,490	6,020A
42	5,710	6,690	6,200A	4,540	4,920	4,730B
$\bar{X}_2$	5,320	6,160	–	4,600	5,190	–
	ANOVA					
P-value (interaction)	.38[c]			.46		
P-value (interval)	<.01			<.01		
P-value (species)	.08			.06		
Harvest interval	2017 ALF	2017 ATF	2017 $\bar{X}_1$	2018 ALF	2018 ATF	2018 $\bar{X}_1$
d	kg ha^{-1}					
Summer						
21	2,310	2,180	2,240B	4,750	3,490	4,120B
28	2,800	2,480	2,640B	4,880	4,160	4,520B
35	4,150	4,280	4,210A	3,550	2,960	3,260C
42	3,870	4,300	4,080A	6,800	6,950	6,650A
$\bar{X}_2$	3,280	3,310	–	4,990	4,280	–
	ANOVA					
P-value (interaction)	.34			.40		
P-value (interval)	<.01			<.01		
P-value (species)	.94			.09		
Harvest interval	2017 ALF	2017 ATF	2017 $\bar{X}_1$	2018 ALF	2018 ATF	2018 $\bar{X}_1$
d	kg ha^{-1}					
Fall						
21	120	130	120D	380	220	300
28	250	500	370C	240	350	300
35	700	580	640B	630	700	670
42	970	1,020	1,000A	410	630	520
$\bar{X}_2$	510	560	–	420	480	–
	ANOVA					
P-value (interaction)	.39			.75		
P-value (interval)	<.01			.06		
P-value (species)	.72			.63		

[a]ALF, alfalfa monoculture; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

FA (although this was not different from the 28 d interval in 2016) (Table 8). In Ohio and North Carolina, the same results were observed during summer, with the 42 d interval generally having greater FA (Table 9 and Table 10). Confirmation by the orthogonal polynomial contrasts showed the cubic relationship of harvest interval with greatest FA for the 42 d interval in Tennessee and Ohio, and in North Carolina in 2017.

TABLE 11 Average crude protein (g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Tennessee

	2016				2017			
Harvest interval	ALF[a]	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
d	g kg^{-1}							
Spring								
21	256	195	196	216A[b]	276	249	214	246A
28	238	175	193	202AB	225	214	172	204B
35	205	181	171	186BC	217	219	176	204B
42	191	162	127	160C	232	212	155	199B
$\bar{X}_2$	223a	178b	171b	–	237a	223a	179b	–
	ANOVA							
P-value (interaction)	.52[c]				.85			
P-value (interval)	<.01				<.01			
P-value (species)	<.01				<.01			
	2016				2017			
Harvest interval	ALF	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
d	g kg^{-1}							
Summer								
21	273Aa	208Ac	224Ab	235	170Bb	185Aab	199Aa	184
28	256Ba	193Bc	222Ab	224	202Aa	175Aa	180Aa	186
35	251Ba	181Cc	212Ab	215	228Aa	194Aab	182Ab	201
42	204Ca	172Cb	196Ba	191	216Aa	202Aa	182Aa	200
$\bar{X}_2$	246	188	213	–	203	188	185	–
	ANOVA							
P-value (interaction)	<.01				.04			
P-value (interval)	NA				NA			
P-value (species)	NA				NA			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.

[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).

[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

In North Carolina, there was a harvest interval effect only during the fall of 2017, with greater FA as the harvest interval increased, as expected. However, fall harvesting can reduce the accumulation of root carbohydrates necessary for regrowth and persistence (Grev et al., 2017; Haagenson et al., 2003). During the fall of 2017, precipitation was lower than in 2018, suggesting that increased rainfall during the months preceding dormancy reduces the harvest interval effect (Table 10). In addition, if we consider that plants are preparing for dormancy in the fall, it is expected that harvest differences will be minor, especially after a year of continuous forage removal, with the maintenance of the vegetative stage combined with higher temperatures (Fuess & Tesar, 1968). The harvest intervals in Tennessee, Ohio, and North Carolina, are not as influenced by seasonality than northern states, as observed by Brink et al. (2010).

4.4 | Nutritive value

In Tennessee, during spring in both years, there was a main effect of species and harvest interval for CP concentration ($P < .01$, Table 11). Greater CP concentrations were observed for ALF and smaller concentrations for ATF, whereas the CP concentration for ABG fluctuated between years. This is because ALF had higher CP than the mixtures with grasses; this result agrees with the findings of Malhi et al. (2002). Moreover, in general, there was a decrease in CP concentration with increased harvest interval (Balde et al., 1993), but this decrease was more pronounced in 2016 than in 2017, probably because of drought stress in 2016 (Hendricks et al., 2020). This trend was confirmed by a linear relationship in 2016 and a quadratic relationship in 2017. In Ohio, both ALF and ABG had a larger CP content than ATF

TABLE 12 Average crude protein (in g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Ohio

	2016				2017			
Harvest interval	ALF[a]	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
d	g kg^{-1}							
Spring								
21	275	265	222	254A[b]	236	242	207	229A
28	233	239	174	215B	194	217	188	199B
35	218	230	170	206B	199	209	174	194B
42	202	215	129	182C	167	193	144	168C
$\bar{X}_2$	232a	237a	173b	–	199a	215a	178b	–
	ANOVA							
P-value (interaction)	.56[c]				.63			
P-value (interval)	<.01				<.01			
P-value (species)	<.01				.01			
	2016				**2017**			
Harvest interval	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
d	g kg^{-1}							
Summer								
21	237	234	225	232	230	228	216	224A
28	212	229	219	220	222	222	192	212AB
35	257	234	240	244	216	217	215	216A
42	202	225	212	213	200	210	192	201B
$\bar{X}_2$	227	231	224	–	219	220	204	–
	ANOVA							
P-value (interaction)	.87				.62			
P-value (interval)	.59				<.01			
P-value (species)	.93				.28			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
b Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

in both years because the legume content was higher for ABG and ALF. These results were also observed by Hendricks et al. (2020),where the addition of alfalfa to bermudagrass increased the CP content of the sward. There was also a decrease in CP concentration as the harvest interval increased (Table 12), which was expected. These results were also confirmed by a linear relationship in 2016 and a cubic relationship in 2017, both leading to the lower CP concentration for the 42-d interval. In North Carolina, the harvest interval was effect was observed only during spring, whereas in 2017, it had the same results as observed for Ohio and Tennessee with decreased CP content with increased harvest interval. Given that in North Carolina only cool-season forages were established, the lack of species differences was predictable. However, in 2018, these results were inconsistent (Table 13), probably because of lower rainfall than in 2017 during the same period.

In the summer, there was a species × harvest interval interaction both years in Tennessee: in 2016, ALF had the greatest CP content (although it was not different from ATF at the 42 d interval), and ABG had a consistently lower CP concentration ($P < .01$, Table 11). Moreover, during the summer of 2016, there was a general decrease in CP concentration with an increase in harvest interval. Since bermudagrass was actively growing during the summer, the reduced concentration of CP was expected in ABG. In addition, CP during this period decreased given its negative correlation with fiber components (Reeves, 1997), which were likely to increase to support plant growth.

The general decrease in CP observed with longer harvest intervals can be attributed to the decrease in the leaf/stem ratio (Albrecht et al., 1987; Yari et al., 2012), given that leaves are known to have a higher CP concentration than stems (Griffin & Jung, 1983), which is expected with maturity (Balde et al., 1993).

TABLE 13 Average crude protein (in g kg^{-1}) during the spring, summer, and fall growing seasons of 2017 and 2018 in North Carolina

	2017			2018		
Harvest interval	**ALF**	**ATF**	**$\bar{X}_1$**	**ALF**	**ATF**	**$\bar{X}_1$**
d	g kg^{-1}					
Spring						
21	281	264	273A[b]	260	235	248A
28	252	255	253B	232	221	226B
35	242	235	238BC	230	226	228B
42	237	230	233C	240	238	239AB
$\bar{X}_2$	253	246	–	240	230	–
	ANOVA					
P-value (interaction)	.57[c]			.50		
P-value (interval)	<.01			.04		
P-value (species)	.21			.30		
	2017			**2018**		
Harvest interval	**ALF**	**ATF**	**$\bar{X}_1$**	**ALF**	**ATF**	**$\bar{X}_1$**
d						
Summer						
21	222	200	218	174	211	195C
28	251	221	236	210	224	217AB
35	236	213	225	223	241	232A
42	215	213	214	187	215	201BC
$\bar{X}_2$	235a	212b	–	199b	223a	–
	ANOVA					
P-value (interaction)	.60			.71		
P-value (interval)	.35			<.01		
P-value (species)	.01			.03		
	2017			**2018**		
Harvest interval	**ALF**	**ATF**	**$\bar{X}_1$**	**ALF**	**ATF**	**$\bar{X}_1$**
d						
Fall						
21	323	274	298	163Cb	239Aa	201
28	318	292	305	200BCa	207Aa	203
35	317	296	306	222ABa	218Aa	220
42	308	270	289	241Aa	242Aa	242
$\bar{X}_2$	316a	283b	–	206	226	–
	ANOVA					
P-value (interaction)	.72			<.01		
P-value (interval)	.54			NA		
P-value (species)	<.01			NA		

[a]ALF, alfalfa monoculture; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.

[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).

[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

In North Carolina during the summer of 2017, ALF showed a higher CP concentration than ATF ($P = .01$, Table 13). However, the opposite occurred during the summer of 2018, with ALF having a lower CP concentration than ATF ($P = .03$, Table 13). Given that rainfall was completely the opposite between both years (Figure 3), it is possible to suggest that higher precipitation is is essential to increase CP content. Moreover, in 2018, a higher CP concentration was observed

TABLE 14 Average neutral detergent fiber (in g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Tennessee

	2016				2017			
Harvest interval	ALF[a]	ABG	ATF	$\bar{X}_1$	ALF	ABG	ATF	$\bar{X}_1$
d	g kg^{-1}							
Spring								
21	317	420	445	394C[b]	285	439	498	407D
28	340	416	446	401BC	333	446	525	434C
35	376	420	469	422B	380	475	535	463B
42	418	446	544	469A	389	526	611	509A
$\bar{X}_2$	362c	425b	476a	–	346c	472b	542a	–
	ANOVA							
P-value (interaction)	.36[c]				.40			
P-value (interval)	<.01				<.01			
P-value (species)	<.01				<.01			
	2016				**2017**			
Harvest interval	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
d	g kg^{-1}							
Summer								
21	286Cb	435Ba	429Aa	383	371	520	524	471
28	299Cc	436Ba	404Bb	379	389	516	537	480
35	340Bc	481Aa	439Ab	420	363	511	579	484
42	402Ac	491Aa	443Ab	445	403	513	560	492
$\bar{X}_2$	332	461	429	–	380c	514b	544a	–
	ANOVA							
P-value (interaction)	<.01				.70			
P-value (interval)	NA				.71			
P-value (species)	NA				<.01			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue;. $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

for the 35 d interval (although it was not different from the 28 d interval) which may be correlated to the higher alfalfa proportion also observed for the 35-d harvest interval. This was also confirmed by a quadratic orthogonal relationship. In the fall of 2017, there was a species effect, with ALF showing more CP than ATF ($P < .01$, Table 13). In the fall of 2018, an interaction between species and harvest interval was observed, with ALF having a lower CP concentration than ATF with the 21 d interval ($P < .01$). However, there were no differences arising from harvest intervals for ATF, but for ALF, less frequent harvesting intervals led to higher CP concentrations (Table 13). Although maturity is normally negatively correlated to CP, forages are usually preparing for dormancy and therefore not actively growing any more leading to a vegetative stage for longer, which explains the increased CP in ALF.

Observations of NDF in Tennessee and Ohio during spring followed, in most instances, the opposite pattern to CP concentration (Table 14 and Table 15), with an effect of species and harvest interval. According to Traxler et al. (1998) as NDF increases, CP decreases, which agrees with our results. More NDF was observed for ATF, followed by ABG, then ALF in both states and seasons. Since ATF was actively growing during the spring (Smith et al., 1992), the increased fiber concentration during this period was expected. In addition, alfalfa alone has the trait of higher CP but a moderate energy value (Veronesi et al., 2010) because of the fibers. Moreover, an increase in NDF can be observed with an increase in harvest interval, because as harvests become less frequent, the NDF content increases as a result of advanced maturity (Balde et al., 1993). These results were confirmed by a linear relationship in 2016 and a quadratic relationship in 2017. In North Carolina, the results were slightly different, with a of species × harvest interval interaction observed in 2017 but only a species effect in 2018 (Table 16).

TABLE 15 Average neutral detergent fiber (in g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Ohio

	2016				2017			
Harvest interval	**ALF**[a]	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	g kg^{-1}							
Spring								
21	261	347	399	335C[b]	269	385	438	364C
28	319	388	460	38B	308	395	449	384C
35	330	391	462	395B	333	425	494	417B
42	400	427	551	459A	420	469	545	478A
$\bar{X}_2$	328c	388b	468a	–	332c	418b	481a	–
	ANOVA							
P-value (interaction)	.69[c]				.53			
P-value (interval)	<.01				<.01			
P-value (species)	<.01				<.01			
	2016				**2017**			
Harvest interval	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**	**ALF**	**ABG**	**ATF**	**$\bar{X}_1$**
d	g kg^{-1}							
Summer								
21	182Cb	388Aa	403Aa	337	330	444	462	412B
28	222Bb	389Aa	415Aa	357	292	419	446	385C
35	298AB	406Aa	406Aa	370	316	438	445	400BC
42	274A,b	398A	410Aa	374	391	459	487	446A
$\bar{X}_2$	274	395	408	–	332c	440b	460a	–
	ANOVA							
P-value (interaction)	.02				.23			
P-value (interval)	NA				<.01			
P-value (species)	NA				<.01			

[a] ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c] NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

In the summer of both years, ATF showed more NDF than ALF and tended to have more for the 42 d harvest interval in Tennessee and Ohio. A species × harvest interval interaction was observed in 2016 with less NDF for ALF and more NDF for ABG (although ABG did not differ from ATF at the 21-d interval). In addition, there was an increase in NDF with increased harvest interval with the exception of ATF at the 21 d interval, which was not different from the 35 or 42 d interval. In 2017, only a species effect was observed, where ALF had the least NDF and ATF had the most (Table 14). In Ohio, similar results were observed, with less NDF for ALF. The interaction between species and harvest interval in 2016 showed a higher NDF concentration for ALF as the harvest interval decreased. However, in 2017, the effect of harvest interval was variable (Table 15). The orthogonal contrasts showed a quadratic relationship, indicating higher NDF concentration for the 42 d interval and decreasing until 28 d, then increasing slightly. In North Carolina, during the summer of both years, the main effects of species and harvest interval were observed for NDF concentration (Table 16), with a lower NDF concentration in ALF. The differences in harvesting interval in the summer of 2017 were variable. However, in 2018, longer harvest intervals led to increased NDF (Table 16): the orthogonal contrast indicated a quadratic relationship, with a constant decrease upt to the 35-d interval and then an increase to the 42-d interval. In the fall of 2017, the main effect of species continued to show, with ALF having more NDF than ATF ($P < .01$) and more NDF being observed for the 42 d interval, although this was not different from the 21d interval ($P = .02$, Table 16). The fact that it did not differ from the 21d harvest interval was probably caused by the higher proportion of weeds, which was also measured during this period. Orthogonal contrasts showed the quadratic relationship of harvest interval, which decreased for the 35 d interval and increased again at 42 d. In the fall of 2018, there was a species × harvest interval interaction, with ALF having less NDF for the 35 d interval than ATF

TABLE 16 Average neutral detergent fiber (in g kg^{-1}) during the spring, summer, and fall growing seasons of 2017 and 2018 in North Carolina

	2017			2018		
Harvest interval	ALF[a]	ATF	$\bar{X}_1$	ALF	ATF	$\bar{X}_1$
d	g kg^{-1}					
Spring						
21	283Bb[b]	449Aa	366	303	448	375
28	319Ab	443Aa	381	297	464	380
35	280Bb	436Aa	358	303	455	379
42	339Ab	455Aa	397	310	447	378
$\bar{X}_2$	305	446	–	302b	453a	–
	ANOVA					
P-value (interaction)	.02[c]			.76		
P-value (interval)	NA			.98		
P-value (species)	NA			<.01		
	2017			**2018**		
	ALF	**ATF**	**$\bar{X}_1$**	**ALF**	**ATF**	**$\bar{X}_1$**
	g kg^{-1}					
Summer						
21	402	525	463A	522	535	528A
28	338	461	400B	435	499	467B
35	383	489	436AB	381	455	418C
42	382	489	436AB	429	508	468BC
$\bar{X}_2$	376b	491a	–	442b	499a	–
	ANOVA					
P-value (interaction)	.96			.33		
P-value (interval)	.02			<.01		
P-value (species)	<.01			<.01		
	2017			**2018**		
	ALF	**ATF**	**$\bar{X}_1$**	**ALF**	**ATF**	**$\bar{X}_1$**
	g kg^{-1}					
Fall						
21	199	441	320AB	552Aa	470Aa	511
28	206	395	301B	488Aa	488Aa	488
35	191	369	280B	332Bb	504Aa	443
42	285	420	352A	337Ba	439Aa	388
$\bar{X}_2$	220b	406a	–	439	475	–
P-value (interaction)	.13			<.01		
P-value (interval)	.02			NA		
P-value (species)	<.01			NA		

[a]ALF, alfalfa monoculture; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

($P < .01$, Table 16). Moreover, harvest interval only affected ALF, with less NDF as it increased ($P < .01$, Table 16).

In Tennessee, there was species × harvest interval interaction during the spring of both years and during the summer of 2016 for lignin concentration (Table 17). However, in the summer of 2017, only the main effects of species and harvest interval occurred (Table 17). During the entire experimental period, ALF generally had a higher lignin concentration than ATF, which was previously observed by Reeves (1987), whereas ABG was variable across seasons and years. For

TABLE 17 Average lignin content (g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Tennessee

	2016				2017			
Harvest interval	**ALF**[a]	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
d	g kg^{-1}							
Spring								
21	49Da[b]	36Bb	32Cc	39	43Ca	45Ba	39Db	43
28	55Ca	35Bc	42Bb	44	52Ba	48Ba	44Cb	48
35	63Ba	37ABc	43Bb	48	63Aa	59Ab	49Bc	57
42	70Aa	42Ac	49Ab	54	64Aa	62Aa	61Aa	62
$\bar{X}_2$	59	38	41	–	56	54	48	–
	ANOVA							
P-value (interaction)	<.01[c]				<.01			
P-value (interval)	NA				NA			
P-value (species)	NA				NA			
	2016				**2017**			
Harvest interval	**ALF**	**ABG**	**ATF**	$\bar{X}_1$	**ALF**	**ABG**	**ATF**	$\bar{X}_1$
d	g kg^{-1}							
Summer								
21	45Ca	35Cb	33Cb	38	52	46	42	46C
28	48Ca	38BCb	37Bb	41	57	49	43	50BC
35	55Ba	41Bb	38Bb	45	58	55	47	53B
42	70Aa	47Ac	53Ab	56	69	65	55	63A
$\bar{X}_2$	53	39	39	–	59a	54b	47c	–
	ANOVA							
P-value (interaction)	<.01				.89			
P-value (interval)	NA				<.01			
P-value (species)	NA				<.01			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals, $\bar{X}_2$, mean of species.

[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).

[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

TABLE 18 Average lignin content (g kg^{-1}) during the spring and summer growing seasons of 2016 and 2017 in Ohio

Harvest interval	2016 ALF[a]	2016 ABG	2016 ATF	2016 $\bar{X}_1$	2017 ALF	2017 ABG	2017 ATF	2017 $\bar{X}_1$
d	g kg^{-1}							
Spring								
21	78Aa[b]	38Cb	28Cc	48	46	61	49	52C
28	80Aa	45Bb	33Bc	53	51	66	56	58B
35	64Ca	47Bb	37Bc	49	57	57	57	62B
42	69Ba	53Ab	43Ac	55	74	74	67	75A
$\bar{X}_2$	73	46	35	–	57b	70a	57b	–
	ANOVA							
P-value (interaction)	<.01[c]				.67			
P-value (interval)	NA				<.01			
P-value (species)	NA				<.01			

Harvest interval	2016 ALF	2016 ABG	2016 ATF	2016 $\bar{X}_1$	2017 ALF	2017 ABG	2017 ATF	2017 $\bar{X}_1$
d	g kg^{-1}							
Summer								
21	79Ba	27Cb	29Bb	45	52	67	57	59C
28	84Aa	41Bb	38Ab	54	49	68	57	58C
35	63Ca	42Bb	39Ab	48	57	77	63	66B
42	68Ca	49Ab	41Ac	53	70	87	71	76A
$\bar{X}_2$	74	40	37	–	57c	75a	62b	–
	ANOVA							
P-value (interaction)	<.01				.39			
P-value (interval)	NA				<.01			
P-value (species)	NA				<.01			

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.
[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).
[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

TABLE 19 Average lignin content (g kg^{-1}) during the spring, summer, and fall growing seasons of 2017 and 2018 in North Carolina

	2017			2018		
Harvest intervals	**ALF**[a]	**ATF**	$\bar{X}_1$	**ALF**	**ATF**	$\bar{X}_1$
d	g kg^{-1}					
Spring						
21	49	67	58B[b]	52Aa	41Ca	46
28	55	68	61A	52Aa	42Ca	47
35	48	66	57B	53Aa	53Ba	53
42	58	69	63A	55Aa	61Aa	58
$\bar{X}_2$	54b	67a	–	53	49	–
	ANOVA					
P-value (interaction)	.10[c]			<.01		
P-value (interval)	<.01			NA		
P-value (species)	<.01			NA		

(Continues)

TABLE 19 (Continued)

Harvest intervals	2017 ALF	2017 ATF	2017 $\bar{X}_1$	2018 ALF	2018 ATF	2018 $\bar{X}_1$
d	g kg^{-1}					
Summer						
21	71	82	76	77Aa	43Bb	60
28	61	85	73	71ABa	49Bb	60
35	72	92	82	63Ba	52ABa	67
42	70	86	78	72ABa	60Ab	66
$\bar{X}_2$	69b	86a	–	71	51	–
	ANOVA					
P-value (interaction)	.54			<.01		
P-value (interval)	.32			NA		
P-value (species)	<.01			NA		
Harvest intervals	**2017 ALF**	**2017 ATF**	**2017 $\bar{X}_1$**	**2018 ALF**	**2018 ATF**	**2018 $\bar{X}_1$**
d	g kg^{-1}					
Fall						
21	41	66	54C	91Aa	33Bb	62
28	48	80	64B	86Aa	37Bb	62
35	46	80	63B	61Ba	58Aa	64
42	60	96	78A	66Ba	62Aa	64
$\bar{X}_2$	49b	81a	–	78	47	–
	ANOVA					
P-value (interaction)	.54			<.01		
P-value (interval)	<.01			NA		
P-value (species)	<.01			NA		

[a]ALF, alfalfa monoculture; ABG, alfalfa–bermudagrass; ATF, alfalfa–tall fescue; $\bar{X}_1$, mean of harvest intervals; $\bar{X}_2$, mean of species.

[b] Means within a column without a common uppercase letter differ in their harvest interval effect ($P < .05$). Means within a row without a common lowercase letter differ in their species effect ($P < .05$).

[c]NA, *P*-value not applicable, since interactions were found. *P*-values were obtained via Tukey's test.

ABG, the median values of NDF and low lignin in the spring of 2016 showed that most of the NDF values are hemicellulose and cellulose; in 2017, we observed an increase in lignification, probably because of the increased maturity of the sward. The 42-d interval consistently showed a higher lignin concentration across all species, seasons, and years (Table 17), which was expected with maturity (Moore & Jung, 2001; Seman et al., 1999). In Tennessee, less lignin content was observed in mixtures than in ALF; therefore, the mixtures of alfalfa and cool- or warm-season grasses decreased the lignin content of the sward, increasing the digestibility of the feed (Buxton & Russell, 1988).

In Ohio, during the spring and summer of 2016, there was an interaction between species and harvest interval (Table 18), with ALF consistently showing more lignification than ABG and ATF; these results were expected, since alfalfa, as a legume, has a higher lignin concentration than cool-season grasses (Spandl & Hesterman, 1997) and many warm-season grasses (Jung et al., 1997). In addition, the lignin content was greater for ALF than for the mixtures; therefore, the lower NDF values observed were driven by the increased lignification. The lignification of ABG and ATF increased as the harvest interval increased, yet in ALF, these results were not consistent. In the spring and summer of 2017, only the main effects of species and harvesting intervals were observed (Table 18). The lowest lignin concentration was observed for ALF and the highest lignin concentration was observed for ABG ($P < .01$); ATF showed intermediate results. The same results were observed in Tennessee, confirming that a mature sward can show an increased accumulation of structural components, such as lignin. Moreover, as harvesting intervals became longer, the lignin concentration increased (Table 18), results that were confirmed by the quadratic relationship in the orthogonal contrasts.

In North Carolina in 2017, only some of the main effects were observed (Table 19), whereas in 2018, a species × harvest interval interaction was observed. During all seasons in 2017, ALF plots showed a lower lignin concentration than ATF ($P < .01$, Table 19). In the spring of 2017, the main effect of harvest interval did not show a clear pattern; during summer, this effect did not occur. However, during the fall of 2017, an increase in lignification was observed as harvest interval increased ($P < .01$, Table 19). Orthogonal contrasts were observed in the spring and fall of 2017, with increased lignin concentrations for the 42 d harvest interval. In 2018, the ATF plots had higher lignin concentrations as the harvest interval increased (Table 19), but when differences were observed for the ALF plots, they were not consistent. No differences in lignin were observed among species in the spring of 2018; however, during summer, ALF had a higher lignin concentration than ATF in most instances ($P < .01$). In the fall, higher lignin concentrations were observed in ALF for shorter harvest intervals ($P < .01$, Table 19). This variation in lignin content across seasons has also been observed in past studies (Buxton & Brasche, 1991; Reeves, 1987).

5 | CONCLUSION

Adopting alfalfa into southern forage programs has benefits such as increased nutritive value and additional increased FA. The 35 d harvest interval provided the greatest FA in all three states, and ATF mixtures are recommended for maximizing FA. Moreover, the 35 d interval allowed plants to maintain vegetative growth while maintaining greater FA and nutritive value. According to the results from Tennessee and Ohio, ABG appeared to be the least productive of the species treatments evaluated. Despite ABG being more productive during summer, it did not show substantial advantages over ATF, even though ATF consisted solely of cool-season species. Nevertheless, studies assessing seeding methods for establishing alfalfa and bermudagrass mixtures are encouraged. In addition, ATF can provide consistently greater nutritive value during the growing season than ABG. According to the results from North Carolina, during fall, there were very few differences in FA, but the frequency of harvesting should be based on the nutritive value instead. Given that weed concentration increased substantially in the second year, alfalfa–grass mixtures have a limited time span of productivity. These harvesting dates were applied to grazing forage programs but could also be adopted in hay production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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