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Harvest interval effects on the persistence and productivity of alfalfa grown as a monoculture or in mixtures in the southeastern United States

Marcia P. Quinby | **Renata L. G. Nave** | **Gary E. Bates** | **David McIntosh**

Dep. of Plant Sciences, Univ. of Tennessee, Knoxville, TN, 37996, USA

Correspondence
Renata L. G. Nave, Dep. of Plant Sciences, University of Tennessee, Knoxville, TN 37996, USA.
Email: rnave@utk.edu

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Abstract

For alfalfa (*Medicago sativa* L.) to be profitable in the southeastern United States, enhanced persistence is required. However, studies assessing alfalfa's persistence in monoculture or mixtures in the region are limited. We aimed to determine the persistence and productivity of alfalfa and alfalfa–grass mixtures subjected to different harvest intervals. Three species combinations were established in 2015: alfalfa (A), alfalfa–tall fescue [*Schenodorus arundinaceus* (Schreb.) Dumort] (ATF), and alfalfa–bermudagrass [*Cynodon dactylon* (L.) Pers.] (AB). These were subjected to four harvest intervals (21, 28, 35, and 42 d) during the 2016, 2017, and 2018 growing seasons. Total forage accumulation (FA) was quantified during 2017 and 2018 and nutritive value and stem density were measured at the first and last harvests in those years. Forage accumulation decreased in all species combinations after 2 yr of management (A: 21 d, –63%; 28 d, –66%; 35 d, –50%; 42 d, –31%; AB: 21 d, –22%; 28 d, –59%; 35 d, –34%; 42 d, –19%; ATF: 21 d, –41%; 28 d, –62%; 35 d, –64%; 42 d, –41%), reflecting decreased alfalfa stem density, especially for A and AB. The differences in alfalfa stem density between the first and last harvests depended on the species. No differences were observed in ATF mixtures but for AB and A, longer harvest intervals had up to 90% fewer stems, thus decreasing alfalfa's persistence in the field. Lower stem density was observed for the 21-d harvest interval; therefore, longer intervals could result in better field performance.

1 | INTRODUCTION

The decision by producers to invest in alfalfa establishment depends on stand persistence, dry matter productivity, and nutritive value. The persistence of legumes, such as alfalfa, relies on the management practices adopted throughout the years, environmental influences, and, if grown in mixtures, the competitiveness between alfalfa and the grass. Alfalfa is a valuable crop throughout the United States; therefore, adequate management and planning is necessary to maintain its economical viability.

In work on alfalfa–grass mixtures, alfalfa is considered the fragile species in the system (Beuselinck et al., 1994). According to Beuselinck et al. (1994), the pattern of defoliation in legumes like alfalfa influences the persistence and therefore, cutting at the vegetative stages will decrease stand persistence compared with harvesting at the reproductive stages. This occurs because when harvests are frequent, the

Abbreviations: AB, alfalfa–bermudagrass; aNDF, amylase-treated neutral detergent fiber; ATF, alfalfa–tall fescue; CP, crude protein; FA, forage accumulation.

TABLE A Useful conversions

To convert Column 1 to Column 2, multiply by	Column 1 suggested unit	Column 2 SI unit
0.304	foot, ft	meter, m
2.54	inch	centimeter, cm (10^{-2} m)
0.405	acre	hectare, ha
0.405×10^3	acre	square meter, sq m
9.29×10^{-2}	square foot, sq ft	squre meter, sq m
28.3	cubic foot, cu ft	liter, L (10^{-3} m^3)
2.83×10^{-2}	cubic foot, cu ft	cubic meter, cu m
2.96×10^{-2}	ounce (liquid), oz	liter, L (10^{-3} m^3)
28.4	ounce (avdp), oz	gram, g
0.454	pound, lb	kilogram, kg
1.12	pound per acre, lb/acre	kilogram per hectare, kg/ha
5/9 (°F – 32)	Fahrenheit, °F	Celsius, °C

plants cannot accumulate enough carbohydrate reserves to support regrowth or cold tolerance (Feltner & Massengale, 1965; Smith, 1969, Rice, Quinsenberry, & Nolan, 1989; Atis, Celiktas, Can, & Yilmaz, 2019). This becomes especially important when managing legume–grass mixtures because harvest frequency can alter the balance of competition among companion species that have different growth patterns.

Alfalfa can experience stand decline when planted in mixtures with tall fescue as the stand matures. In the study of Aponte, Samarappuli, and Berti (2019), it was observed that alfalfa lowers its presence in mixtures as stands age. The extent of this depends on the alfalfa cultivar used and the management practices implemented (Smith et al., 1992). However, studies have shown that mixtures of alfalfa and tall fescue increased FA compared with tall fescue in monoculture fertilized with 119 lb N acre^{-1} (Lauriault, Guldan, & Martin, 2003). According to Brophy and Heichel (1989), alfalfa can release up to 4.5% of its N to the root zone with high transferability. Therefore, alfalfa supplies N through biological N fixation, decreasing reliance on synthetic fertilizers (Waldron, Peel, Larson, Mott, & Creech, 2017). In addition, legume–grass mixtures are known to benefit soil health (Dakhal & Islam, 2018). Nitrogen fixation by associated legumes is also important for other grasses such as bermudagrass, which is highly responsive to N (Massey et al., 2011).

Brown and Byrd (1990) compared the FA of alfalfa and bermudagrass in monoculture and in mixtures subjected to three levels of N fertilization. Their results suggested that mixing alfalfa with bermudagrass provides similar FA to alfalfa in monoculture and to fertilized bermudagrass monocultures. However, Stringer, Khalilian, Undersander, Stapleton, and Bridges (1994) observed that alfalfa mixed with bermudagrass resulted in decreased bermudagrass vigor as a result of shading and competition for water.

Studies assessing alfalfa persistence in monoculture or mixtures in the southeastern United States are limited. However, livestock producers are interested in whether alfalfa can persist long enough in the region so that costs of production, including seed purchase, are worthwhile. Therefore, the objective of this study was to evaluate alfalfa in monoculture and in mixtures with either tall fescue or bermudagrass subjected to different harvest intervals over 3 yr. We hypothesized that adjusting the harvest interval would allow increased persistence of alfalfa, offering the potential to maximize profitability for hay or livestock producers.

Core Ideas

- Alfalfa can be grown with perennial grasses in the southeastern United States.
- Management practices are crucial for alfalfa persistence and productivity in mixtures.
- Longer harvest intervals are favorable to persistence in perennial grass mixtures.

2 | SITE DESCRIPTION

This study was conducted at the Plateau AgResearch and Education Center in Crossville, TN (36°01′N, 85°12′W) from 2015 to 2018 and sampled from May 2017 to Sept. 2018. Soil conditions at the location were Lily loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults), which is fine-loamy residuum weathered from sandstone on 2 to 6% slopes and well drained, with 21 to 39 inches to lithic bedrock (NRCS, 2018). Soil samples were collected at 0- to 6-inch depth and sent to the Tennessee Soil, Plant, and Pest Center (Hanlon & Savoy, 2007). The initial soil nutrient levels at the experiment site based on Mehlich 1 extractions were: P, 69 lb acre^{-1}; K, 221 lb acre^{-1}; Ca, 1324 lb acre^{-1}; Mg, 56 lb acre^{-1}; the pH was 5.5.

Three experiments (one for each species combination) were conducted in experimental units that were 10 by 20 ft in area and arranged in a completely randomized design with four replications. All species combinations were subjected to four harvest intervals, totaling 16 experimental units for each experiment. The species were grazing-tolerant 'Ameristand 403T Plus' grown in monoculture (A) or in a mixture with the novel endophyte 'Texoma Max Q II' tall fescue (ATF) or 'Vaughn's #1′ bermudagrass (Vaughn, 1994) (AB). The four harvest intervals were 21, 28, 35, and 42 d. All plots were established in 2015 and harvest intervals were imposed for each species combination in May 2016. However, sampling and data collection for the current experiment occurred during the 2017 and 2018 growing seasons (2 and 3 yr, respectively, after initial establishment).

On 16 July 2015, the ground was tilled and fertilized with 60 lb N acre^{-1} (34–0–0 N–P–K) followed by establishment of bermudagrass via vegetative propagation with small bales of stem cuttings at a rate of 12 ft^3 acre^{-1}. On 4 Sept. 2015, alfalfa was drill seeded with a 10-ft ft no-till drill model (Great Plains, Salina, KS) at a spacing of 7 inches between rows, across the entire experimental area (including the plots with established bermudagrass) at 15 lb acre^{-1}. Immediately after alfalfa seeding, tall fescue was drill-seeded with the same drill at 10 lb acre^{-1} to all ATF plots, which was made by following the same drilling path made for alfalfa seeds ensuring that the fescue seeds would be seeded into the same previously drilled rows containing alfalfa. On 7 Oct. 2015, 60 lb N acre^{-1} (34–0–0 N–P–K) was applied to the ATF plots as a single application to ensure establishment. On 26 Oct. 2015, the entire experimental area was treated with lime at 5442 lb acre^{-1} according to the soil testing recommendations for all three species combinations. On 2 Feb. 2016, 24 Feb. 2017, and 15 Feb. 2018, B was applied at 2 lb acre^{-1} to the entire experimental area, in line with soil test recommendations.

On 8 Mar. 2016, alfalfa was reseeded on the AB plots at 15 lb acre^{-1} with a Hege small tube drill because of the lower than expected density from the previous seeding. On 25 May 2016, 2,4-DB 200 [a.i.: 4-(2,4-dichlorophenoxy)butanoic acid; Butyrac Agrisolutions, WindField, MN] was applied to all plots to control broadleaf weeds at 63 oz acre^{-1}. Because of the dry conditions in 2016 (Figure 1), on 23 and 24 Sept. 2016, an irrigation gun on a reel system was used over the entire experimental area for a total of 0.98 inches. Chlorpyrifos [a.i.: chlorpyrifos: *O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate; Lorsban DowAgrosciences Canada Inc.] was applied on 6 Apr. 2017 to control alfalfa weevil (*Hypera postica*) and on 9 May 2017 to control potato leafhopper (*Empoasca fabae)* at a rate of 14 oz acre^{-1} per application. According to the 2018 soil testing recommendations, 178 lb K acre^{-1} was applied to all plots

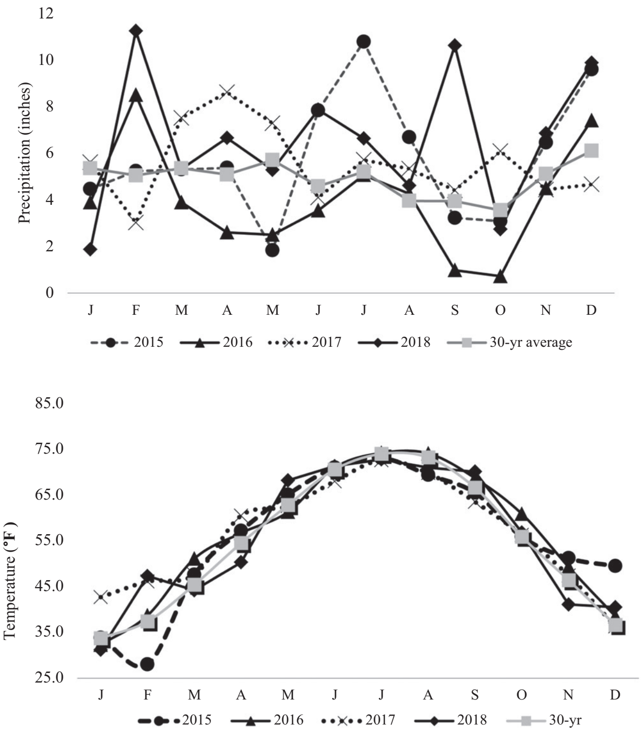

FIGURE 1 Weather for Crossville, TN, reported as monthly averages based on daily records, including the past 30-yr and averages for 2015, 2016, 2017, and 2018

in the three experiments. It was split into two applications (11 Apr. and 13 June 2018). Furthermore, on 13 June 2018 chlorpyrifos was applied to control alfalfa weevil and alfalfa leafhopper (14 oz acre^{-1}) and 2–4 DB 200 (14 oz acre^{-1}) was applied to control broadleaf weeds (white clover [*Trifolium repens* L.]). Clethodim herbicide [a.i.: clethodim (2-[(*E*)-*N*-[(*E*)-3-chloroprop-2-enoxy]-*C*-ethylcarbonimidoyl]-5-(2-ethylsulfanylpropyl)-3-hydroxycyclohex-2-en-1-one); SelectMax Valent, Walnut Creek, CA) was applied to A plots (32 oz acre^{-1}) to control grasses (tall fescue and crabgrass [*Digitaria sanquinalis* (L.) Scop]).

2.1 | Measurements

The center strip in each experimental plot was harvested with a 3-ft-wide Carter forage harvester (Carter, Brookston, IN) at a stubble height of 4 inches. The border material of each plot was removed after each harvest. All species followed the same harvesting schedule. The dates of the imposed harvests are displayed in Table 1.

During each harvest in 2017 and 2018, the forage wet weight was recorded. A subsample was then collected from the harvested material and the wet weights of the subsamples were recorded. Forage samples were dried at 140 °F to a constant weight (~72 h) and the dry weights were used to calculate FA. Samples were then ground to pass through a

TABLE 1 Dates of imposed harvest intervals in 2016, 2017, and 2018 across treatments

	Harvest interval			
Year	**21 d**	**28 d**	**35 d**	**42 d**
2016	3 May	10 May	17 May	24 May
	24 May	7 June	21 June	8 July
	14 June	8 July	26 July	16 Aug.
	8 July	3 Aug.	30 Aug.	–
	26 July	30 Aug.	–	–
	16 Aug.	–	–	–
2017	2 May	9 May	16 May	23 May
	23 May	6 June	20 June	5 July
	13 June	5 July	25 July	15 Aug.
	5 July	1 Aug.	29 Aug.	–
	25 July	29 Aug.	–	–
	15 Aug.	–	–	–
	5 Sept.	–	–	–
2018	1 May	9 May	15 May	22 May
	22 May	5 June	19 June	3 July
	13 June	3 July	24 July	14 Aug.
	3 July	31 July	28 Aug.	–
	24 July	29 Aug.	–	–
	14 Aug.	–	–	–
	4 Sept.	–	–	–

1-mm sieve with a Wiley Mill (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA).

Samples from the first and last harvest of 2017 and 2018 were analyzed for crude protein (CP) and amylase-treated neutral detergent fiber (aNDF) via near infrared reflectance spectroscopy (Unity SpectraStar XL-R, Unity Scientific, Milford, MA). Samples collected in 2016 were also analyzed but not reported in this manuscript. Spectra for the forage nutritive analyses were standardized and the 2016–2017 Grass Hay and Legume Hay equations developed by the Near Infrared Reflectance Spectroscopy Forage and Feed Consortium (Hillsboro, WI) were used to predict the nutritive value. The software used was Infostar version 3.11.3 3 (Unity Scientific, Milford, MA). The Global *H* statistical test compared each sample against the population structure of the prediction equation's calibration set to ensure validity within the parameters of the equation (Murray & Cowe, 2004). Alfalfa stem density was quantified in each experimental unit at the first and last harvest of 2017 and 2018 in two randomly placed 1-ft^2 quadrats.

2.2 | Statistical analysis

Differences between least square means by harvest interval for cumulative FA, alfalfa stem density, and total forage CP and aNDF for each forage treatment were evaluated via the PROC MIXED procedure, adjusted via the Holm–Bonferroni method for least square mean separations in SAS (SAS for Windows version 9.4, SAS Institute, Cary, NC). Response variables (FA, alfalfa stem density, CP, and aNDF) were considered to be dependent. Year, harvest interval, and harvest day (Table 1) were considered to be fixed effects; replication was considered to be a random effect. The results are displayed separately for each forage treatment. All results were evaluated for significance at $P < .05$.

2.3 | Environmental conditions

The annual temperature was 2% above the 30-yr average in 2015, 3% above the 30-yr average in 2016, 2% above the 30-yr average in 2017, and 1% above the 30-yr average in 2018. The annual precipitation was 18% above the 30-yr average in 2015, 19% below the 30-yr average in 2016, 13% above the 30-yr average in 2017, and 34% above the 30-yr average in 2018. Conditions during each harvest were as follows: May to September temperatures were 0.6% above the 30-yr average in 2016, 3% below the 30-yr average in 2017, and 2% above the 30-yr average in 2018. Precipitation from May to September was 30% below the 30-yr average in 2016, 14% above than the 30-yr average in 2017, and 49% above than the 30-yr average in 2018 (Figure 1).

2.4 | Alfalfa monoculture

2.4.1 | Stem density

There was not a three-way interaction for stem density of alfalfa monocultures for harvest day × harvest interval × year ($P = .21$) but there was a two-way interaction of harvest day and harvest interval ($P = .02$). There were no differences among harvest intervals in the first harvest, but for the last harvest, the 42-d interval and the 35-d interval showed greater stem counts but the 35-d interval was not different from the 28-d interval (Table 2). These results suggest that lower harvesting frequencies allow for a greater stem count in A, which can be correlated with an increased persistence of alfalfa in the field. In addition, it is possible to observe that for the 21-, 28-, and 35-d intervals, stem density at the first and last harvest was different, whereas for the 42-d interval, no differences were observed (Table 2)($P = .9005$).

For harvests that occur more frequently, the number of stems was greater at the beginning of the season, probably because of the rapid mobilization of root carbohydrates after dormancy, which led to an increase in plant growth and branching (Li, Volnec, Joern, & Cunningham, 1996). Meanwhile, with the 42-d interval, there were no differ-

TABLE 2 Alfalfa stem density (±SE) in alfalfa monoculture at the first and last harvests with four harvest intervals during the 2017 and 2018 growing seasons

Harvest interval	First harvest	Last harvest	Difference between first and last harvest
d	number of alfalfa stems per ft^2		
21	44 ± 9[a]	16 ± 4 c	*
28	59 ± 8	30 ± 9 bc	*
35	65 ± 6	44 ± 8 ab	*
42	65 ± 6	66 ± 9 a	ns

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.
*Significant at the .05 probability level between the first and last harvest by harvest interval treatment. ns, not significant.

TABLE 3 Total forage accumulation (±SE) of alfalfa monoculture at four harvest frequencies during the 2017 and 2018 growing seasons

Harvest interval	2017	2018	Year differences
d	———lb acre^{-1}———		
21	5,000 ± 143[a] b	1,831 ± 528 c	*
28	6,969 ± 309 a	2,312 ± 359 bc	*
35	7,176 ± 788 a	3,562 ± 199 ab	*
42	6,395 ± 165 ab	4,379 ± 186 a	*

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.
*Significant at the .05 probability level between 2017 and 2018 by harvest interval treatment.

ences between the first and last harvests. This occurred because alfalfa plants require a longer period to restore root carbohydrates between harvests. It is well established that frequent harvests are deleterious to forage root mass (Feltner & Massengale, 1965; Smith, 1969; Rice et al., 1989). In addition, Cowett and Sprague (1962) observed that harvesting alfalfa at more mature stages leads to an increased number of stems compared with immature plants. This is likely to be another reason for the lack of differences at the first harvest as opposed to the last harvest of the season.

2.4.2 | Forage accumulation

There was a harvest interval × year interaction ($P = .02$) for FA. In 2017, the 21- and 42-d intervals had the lowest FA (Table 3). It is likely that the similarity between the 21- and 42-d intervals is related to forage maturity, given that frequent harvests do not allow for sufficient alfalfa regrowth, whereas longer periods between cuttings enable alfalfa to reach maturity, resulting in loss of leaves, decreasing the leaf/stem ratio and consequently reducing FA. In addition, with advanced maturity, increased shading of the bottom leaves can also decrease the leaf/stem ratio (Fuess & Tesar, 1968; Marten & Hovin, 1980).

In 2018, there was an increase in FA with lower harvesting frequencies (Table 3). If we consider that all harvest intervals, FA in 2017 was greater than 2018 (Table 3), it is likely that weed species biomass (mainly crabgrass and white clover) in 2018 (visual observations, data not shown) made up a greater portion of the FA. According to Berti, Nudell, and Meyer (2012), as the stands gets older, stem density decreases regardless of the management adopted, which also explains the decrease FA in 2018. Similar results were reported by Berti and Samarappulli (2018), as stands aged, the plant and stem density decreased.

In addition, the increased competition resulted in the overall decrease of alfalfa FA, thus allowing the harvesting interval effects to be more pronounced. Intense harvest schedules combined with weed species competition were highly damaging to alfalfa, suggesting that increased maturity allows alfalfa to better compete with future weed species infestation. These results agree with Reynolds (1971), who observed similar results in Tennessee, in which shorter harvest intervals led to lower yield, explained by reduced alfalfa competitiveness at earlier stages of maturity. It has been reported that the use of reduced lignin cultivars can allow producers to use alfalfa at longer harvesting frequencies to maintain yield and forage nutritive value (Arnold et al., 2019), which could potentially lead to decreased weed biomass.

2.4.3 | Forage nutritive value

There was a three-way interaction of year × harvest day × harvest interval ($P < .01$) in CP and aNDF values. After the years were analyzed separately, there was a two-way interaction of harvest day × harvest interval ($P < .01$) in both years for CP and aNDF (Table 4). In 2017, there were no differences in the concentration of CP among harvest intervals at the first harvest. However, the last harvest for the 21-d interval had the lowest mean CP concentration, which did not differ from the 28-d interval. It would be expected that as maturity increased with longer harvest intervals, CP concentration would decrease (Albrecht, Wedin, & Buxton, 1987; Sheaffer, Lacefield, & Marble, 1988; Sheaffer et al., 2000); however, we observed that the number of alfalfa stems increased with increased harvesting interval (Table 2), explaining the greater CP concentration for longer harvest intervals. A similar pattern was observed for aNDF concentration (Table 4), which is in agreement with past literature showing that CP and aNDF are negatively correlated (Reeves, 1997). In 2017,

TABLE 4 Concentration of crude protein and neutral detergent fiber (±SE) of alfalfa monoculture at the first and last harvests with four harvest frequencies during the 2017 and 2018 growing seasons

Year	Harvest interval	First harvest	Last harvest	Difference between first and last harvest
	d	Crude protein (%)		
2017				
	21	23.4 ± 0.2[a]	14.6 ± 1.3 b	*
	28	20.9 ± 0.2	16.8 ± 2.3 ab	ns
	35	21.6 ± 0.5	21.7 ± 1.9 a	ns
	42	23.3 ± 0.3	21.0 ± 1.0 a	ns
2018				
	21	15.0 ± 0.9 b	12.7 ± 0.4 b	ns
	28	21.0 ± 0.7 a	12.1 ± 0.6 b	*
	35	23.7 ± 0.4 a	15.3 ± 1.7 ab	*
	42	22.6 ± 0.6 a	18.9 ± 1.5 a	*
		Neutral detergent fiber (%)		
2017				
	21	30.6 ± 0.4	48.8 ± 2.7 a	*
	28	34.0 ± 0.6	45.1 ± 4.2 ab	*
	35	37.4 ± 0.9	37.4 ± 3.2 b	ns
	42	38.9 ± 0.8	37.8 ± 1.4 b	ns
2018				
	21	39.7 ± 1.2	51.3 ± 1.8 a	*
	28	34.0 ± 1.1	56.5 ± 2.2 a	*
	35	30.9 ± 0.5	47.4 ± 3.2 ab	*
	42	36.0 ± 0.9	38.5 ± 3.6 b	ns

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.
*Significant at the.05 probability level between the first and last harvest by harvest interval treatment and year. ns, not significant.

the difference between the first and second harvests was only observed at the 21-d interval for CP and at the 21 and 28-d intervals for aNDF (Table 4). These results could be attributed to the fact that alfalfa's presence was greater in less frequent harvests (Table 2), maintaining greater nutritive value.

In 2018, during the first harvest, the 21-d interval had the lowest mean CP concentration, though for the last harvest, the 21- and 28-d intervals had the lowest mean CP concentrations (Table 4). It is likely that with an increase in observed weed biomass, alfalfa remained vegetative for a longer period, thus maintaining a greater concentration of CP. However, aNDF showed no differences among harvest intervals at the first harvest of 2018 but during the last harvest, it followed the same pattern for CP. Of the nutritive value variables, changes in CP concentration are more pronounced in forage legumes, which can explain the lack of aNDF differences at the first harvest of 2018 compared with CP concentration during the same period. In addition, grazing-tolerant cultivars of alfalfa (such as the cultivar used in this study) have lower aNDF then alfalfa cultivars that are not bred for grazing, as seen by Kallenbach, Nelson, and Coutts (2002), which could explain the inconsistent results observed in this study.

Differences between the first and last harvests in 2018 were observed for the 28-, 35-, and 42-d intervals, with a greater concentration of CP in the first harvest than in the last (Table 4). This is probably caused by the increased maturity of alfalfa plants, whereas the 21-d interval remained vegetative in the field, maintaining similar CP concentration. Neutral detergent fiber showed differences in 2018 for all harvest intervals between the first and last harvests, with the exception of the 42-d interval. Since there were no stem differences between the first and last harvests' 42-d interval (Table 2), it is likely that the lack of differences for aNDF was caused by the stem count observed.

The results observed thus far showed that longer harvest intervals lead to better nutritive value; however, it has been well established that as maturity increases, the nutritive value declines (higher CP and lower aNDF). It is believed that the results observed from this study represent the effects that weed species can have in response to the harvest intervals.

TABLE 5 Alfalfa stem density (±SE) in alfalfa–bermudagrass mixtures at the first and last harvests with four harvest intervals during the 2017 and 2018 growing seasons

Year	Harvest interval	First harvest	Last harvest	Difference between first and last harvest
	d	———Number of alfalfa stems per ft^2———		
2017				
	21	56 ± 6[a]	11 ± 4 b	*
	28	44 ± 11	32 ± 8 a	ns
	35	55 ± 5	52 ± 13 a	ns
	42	52 ± 8	51 ± 12 a	ns
2018				
	21	13 ± 2 b	1 ± 1 b	*
	28	37 ± 7 a	17 ± 3 a	*
	35	49 ± 5 a	16 ± 3 a	*
	42	49 ± 8 a	25 ± 8 a	*

[a]Means within a column without a common lower letter differ ($P < .05$) according to Tukey's test.
*Significant at the .05 probability level between the first and last harvest by harvest interval treatment and year. ns, not significant.

Therefore, management strategies to suppress weeds should be investigated when growing alfalfa.

2.5 | Alfalfa–bermudagrass mixtures

2.5.1 | Stem density

There was a three-way interaction for harvest day × harvest interval × year ($P < .01$) for stem density in AB mixtures; therefore, harvest day and harvest interval were analyzed by year. In 2017, there was harvest day × harvest interval interaction in 2017 ($P = .04$) and in 2018, differences were seen in the main effects of harvest day ($P < .01$) and harvest interval ($P < .01$).

In 2017, there were no differences among treatments at the first harvest; however, the 21-d interval had the fewest stems at the last harvest (Table 5). Alfalfa can shade bermudagrass and decrease grass vigor (Stringer, Khalilian, Undersander, Stapleton, & Bridges, 1994), so the longer the harvest interval, the greater the shading. In addition, in the comparison between the first and last harvests, only the 21-d interval showed differences (Table 5), with a greater number of stems at the first than at the last harvest. This was expected because early in the season, the sward has had enough time to recover from the past growing season, whereas later in the season, the recovery period was only 21 d.

In 2018, the 21-d interval had the lowest stem count but all other harvest frequencies did not differ (Table 5). The lower number of stems associated with more frequent harvests was attributed to the excessive removal of photosynthetic leaves at harvest, which decreases stem density (Cuomo, Anderson, & Young, 1998; Tracy & Jones, 2018; Chatterton, Carlson, Hart, & Hungerford, 1974). The appearance of new stems is associated with activation of the axillary buds of the first leaf; therefore, the number of stems is strongly associated with the number of leaves present in the sward (Gastal & Lemaire, 2015). In addition, frequent harvests will deplete the root carbohydrates, limiting alfalfa growth and competitiveness with other species when in a mixture. Meanwhile, the 21-d interval allowed bermudagrass to grow without shading by alfalfa, increasing grass competitiveness.

In 2018, all harvest intervals had greater stem density at the first harvest than at the last (Table 5). Considering that this pattern was not observed in 2017, this suggests that the persistence of alfalfa in AB mixtures tends to decrease in the third year of applied management, independent of the harvest interval. It was observed by Aponte et al. (2019) that alfalfa's presence in grass–legume mixtures decreased as stands aged, which was seen in our study. Therefore, additional management strategies are needed to maintain a desired alfalfa percentage in the mixture, such as planting alfalfa at a wider row spacing (27 inches) (Haby, Davis, & Leonard, 1999) rather than drilled throughout the entire plot. Interseeded alfalfa at a wide row spacing will allow bermudagrass to compete in these plots (Stringer et al., 1994), maintaining the ideal legume/grass ratio, prolonging the persistence of these species, and avoiding weed infestations.

2.5.2 | Forage accumulation

There was a harvest interval × year interaction ($P < .01$) in FA. In 2017, the 21-d interval had the lowest FA out of all the

TABLE 6 Total forage accumulation (±SE) of alfalfa–bermudagrass mixtures at four harvest frequencies during the 2017 and 2018 growing seasons

Harvest interval	2017	2018	Year differences
d	———lb $acre^{-1}$———		
21	4,341 ± 254[a] b	3,374 ± 177 bc	Ns
28	6,353 ± 384 a	2,619 ± 259 c	*
35	6,975 ± 505 a	4,624 ± 254 ab	*
42	6,729 ± 511 a	5,436 ± 268 a	*

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.
*Significant at the .05 probability level between 2017 and 2018 by harvest interval treatment. ns, not significant.

TABLE 7 Concentration of crude protein and neutral detergent fiber (±SE) of alfalfa–bermudagrass mixtures at the first and last harvests with four harvest frequencies during the 2017 and 2018 growing seasons

Harvest interval	First harvest	Last harvest	Difference between first and last harvest
d	———Crude protein (%)———		
21	17.7 ± 1.2[a] b	14.8 ± 0.6 ab	ns
28	20.4 ± 0.9 ab	13.4 ± 1.6 b	*
35	22.0 ± 0.6 a	16.1 ± 1.0 ab	*
42	20.6 ± 0.5 ab	18.1 ± 0.9 a	ns
	———Neutral detergent fiber (%)———		
21	46.0 ± 1.2 ab	56.4 ± 1.6 a	*
28	44.3 ± 1.6 b	56.0 ± 2.2 a	*
35	45.9 ± 1.3 ab	51.4 ± 1.6 ab	*
42	51.0 ± 0.9 a	48.9 ± 0.8 b	ns

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.
*Significant at the .05 level of probability between the first and last harvest by harvest interval treatment. ns, not significant.

harvest frequencies (Table 6). The 28-, 35-, and 42-d intervals did not show any differences in FA. These results suggest that when alfalfa plants are not able to regrow and recover root carbohydrates (Davis, McGraw, Beuselinck, & Roberts, 1995; Dhont, Castonguay, Nadeau, Bélanger, & Chalifour, 2002), it can result in decreased FA and persistence over time.

In 2018, harvest intervals had a stronger effect, because the 42-d frequency had high FA and more variable results based on the remaining harvest intervals (Table 6). These results are supported by the fact that the depletion of root carbohydrates by frequent harvesting affects FA accumulation. In our results, the 35-d interval did not differ from the 21-d interval, but it was greater than the 28-d interval (Table 6). This is probably because of the increased number of weeds observed in the 21-d interval (visual observations, data not shown). Similarly, the 21-d interval was the only harvest interval in which there were no differences between years, though all the other harvest intervals had reduced FA in 2018 (Table 6). These results are possibly because frequent harvests were a detriment to the AB mixture, enabling the establishment of weeds. The appearance of weed species in the frequently harvested plots can appear to maintain the FA from previous years. Therefore, most of the FA reported in frequently harvested plots accounts for the presence of these weed species. In addition, most producers growing legume–grass mixtures are hesitant to use herbicides, so allowing longer harvest intervals in these mixed systems are the best recommended management.

2.5.3 | Forage nutritive value

There was a harvest day × harvest interval interaction ($P < .05$) and no effects of year for CP and aNDF in AB plots. Therefore, yearly results were combined for CP and aNDF. For the first harvest, greater CP content was shown for the 35-d interval but were not different between the 28- and 42-d intervals. For the last harvest, the 42-, 35-, and 21-d intervals did not differ in CP concentration; the 28-d interval had lower CP concentration but was not different from the 21- and 35-d intervals (Table 7). These inconsistent results were also shown for aNDF concentration. It is known that as CP concentration

TABLE 8 Alfalfa stem density (±SE) in alfalfa–tall fescue mixtures at the first and last harvests with four harvest intervals during the 2017 and 2018 growing seasons

Year	Harvest interval	First harvest	Last harvest
	d	number of alfalfa stems per ft^2	
2017			
	21	36 ± 2[†]	29 ± 11
	28	39 ± 5	26 ± 3
	35	36 ± 3	33 ± 5
	42	28 ± 4	43 ± 12
2018			
	21	6 ± 1	3 ± 2
	28	13 ± 3	16 ± 5
	35	11 ± 1	8 ± 4
	42	21 ± 2	16 ± 2

decreases, aNDF concentration increases. This is because of the advanced stages of maturity, where plants increase their fiber components to maintain tissues that will support plant growth and structure (Wiersma et al., 1998). This occurs in all forage plants, including legumes and grasses, but advanced maturity tends to decrease the nutritive value of stems more pronouncedly in forage legumes than in grasses (Corbin, Nave, Bates, Butler, & Hawkins, 2018). This is probably a result of the similar inconsistencies in the differences among harvest intervals for AB plots in FA (Table 6) and stem density (Table 5) (Table 6).

However, if we consider differences in the nutritive value between the first and last harvests for each harvest interval (Table 7), only the 28-d and 35-d intervals showed differences in CP. This result indicates that when the forages are kept vegetative (21-d interval) or have reached advanced maturity (42-d interval), the concentration of CP does not change throughout the growing season. Differences in CP will be more evident in plants that are actively growing tissues, as observed for the 28- and 35-d intervals (Table 7). For aNDF, all harvest intervals showed differences between the first and last harvest, with the exception of the 42-d interval. Differences in fiber concentration are more likely to be noticeable with advanced maturity.

2.6 | Alfalfa–tall fescue mixtures

2.6.1 | Stem density

There was only a year effect in ATF mixtures ($P < .01$) for stem density, with 2017 having more stems than 2018 (Table 8). In 2017, the mean number of stems across all harvest intervals was 34 ± 3 per ft^2 compared with 12 ± 1 per ft^2 in 2018. These results document the overall reduction of alfalfa in ATF mixtures 3 yr after the management was applied, which is associated with the decreased persistence of the legume when grown with a grass or as a monoculture (Table 2). These results are in agreement with the findings of Pearen and Baron (1996), who observed that as the stand ages, the density of alfalfa stems is reduced, thus allowing tall fescue to reach maturity and dominate resource availability, independently of how frequently harvest occurs. There were also no differences between the first and last harvests for all harvest intervals, indicating that tall fescue is highly competitive throughout the entire growing season. Frequent harvests allow forage species to maintain the sward at a vegetative stage, which is especially beneficial for tall fescue because of its rapid FA during early spring and late summer (Nave, Sulc, Barker, & St-Pierre, 2014).

2.6.2 | Forage accumulation

There was a harvest interval × year interaction ($P < .01$) for FA in the ATF experiment (Table 9). In 2017, as expected, the 21-d interval had the lowest FA, whereas the 42-d interval and the 35-d interval showed the greatest FA. However, in 2018, the 42-d interval had the greatest FA (Table 9). These results suggest that after establishment, as time passes, ATF mixtures can benefit from longer harvesting frequencies in order to have adequate FA in the field, especially when the goal is to maintain alfalfa persistence in the mixture, based on the number of alfalfa stems observed on Table 8. This result is in agreement with Pomerleau-Lacasse et al. (2019), who observed that harvesting alfalfa–grass mixtures at more mature stages increased dry matter accumulation and persistence of the mixture in the field.

TABLE 9 Total forage mass (±SE) of alfalfa–tall fescue mixtures at four harvest frequencies during the 2017 and 2018 growing seasons

Harvest interval	2017	2018	Year differences
d	lb acre^{-1}		
21	4,200 ± 228[a] c	2,458 ± 359 b	*
28	6,197 ± 614 b	2,337 ± 186 b	*
35	7,385 ± 422 ab	2,614± 211 b	*
42	7,949 ± 278 a	4,688 ± 229 a	*

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.

[*]Significant at the .05 probability level between 2017 and 2018 by harvest interval treatment.

TABLE 10 Concentration of crude protein and neutral detergent fiber (±SE) of alfalfa–tall fescue mixtures at the first and last harvests with four harvest frequencies during the 2017 and 2018 growing seasons

Year	Harvest interval	First harvest	Last harvest	Difference between first and last harvest
	d	——————Crude protein (%)——————		
	21	11.7 ± 0.5[a] b	15.9 ± 0.7	*
	28	12.3 ± 0.3 b	15.5 ± 0.5	*
	35	11.9 ± 0.9 b	14.5 ± 0.7	*
	42	14.0 ± 0.8 a	14.8 ± 0.7	ns
		———Neutral detergent fiber (%)———		
2017				
	21	60.3 ± 0.9	56.8 ± 1.1	ns
	28	60.4 ± 0.9	60.7 ± 1.6	ns
	35	59.7 ± 1.1	61.2 ± 0.9	ns
	42	61.1 ± 0.5	57.8 ± 0.4	ns
2018				
	21	51.0 ± 1.4 c	54.8 ± 0.9	ns
	28	55.2 ± 0.9 bc	52.2 ± 1.2	ns
	35	62.1 ± 1.0 a	57.4 ± 1.3	*
	42	60.3 ± 1.8 ab	54.5 ± 2.1	*

[a]Means within a column without a common lowercase letter differ ($P < .05$) according to Tukey's test.

*Significant at the .05 level between the first and last harvests by harvest interval treatment. ns, not significant.

In addition, all the harvest intervals showed decreased FA between 2017 and 2018 (Table 9), confirming that ATF's productivity decreases with time. According to the decreased number of alfalfa stems in these plots (Table 8), it is likely that the FA observed in 2018 is dominated by tall fescue, which outcompeted alfalfa during the third year after establishment.

2.6.3 | Forage nutritive value

There was a two-way interaction of harvest day × harvest interval ($P < .01$) for the CP values and therefore, the years were combined. However, for aNDF, a three-way interaction of year × harvest day × harvest interval ($P < .01$) was observed, so years were separated for the NDF analysis.

Greater mean CP was attributed to the 42-d interval at the first harvest; however, no differences were observed at the last harvest (Table 10). For aNDF, no differences were observed in 2017; in 2018, differences among treatments were only observed in the first harvest, with the 35-d interval having the greatest NDF (Table 10). Although there were no differences among harvest intervals regarding the number of stems (Table 8), it is likely that in this mixture, tall fescue outcompetes alfalfa, thus decreasing alfalfa density (Smith et al, 1992).

Differences between the first and last harvest were found in CP for the 21-, 28- and 35-d intervals (Table 10). Overall, the nutritive value of ATF was greater with the presence of alfalfa than in tall fescue monocultures (Nave et al., 2014). According to a study conducted by Read, Lang, and Aiken (2017), tall fescue pastures that were fertilized with 150 lb N acre^{-1} had a range of 7 to 14% CP, which is still, in most instances, lower than the CP values found in this study. The results suggest that the addition of alfalfa to tall fescue mixtures generates an overall increase in nutritive value.

No differences in aNDF were found between the first and last harvests during 2017 (Table 10), whereas in 2018, the first harvest showed greater aNDF for the 35- and 42-d intervals. Tall fescue increases fiber content after dormancy, which could explain the differences in aNDF content throughout the growing season with advanced maturity.

3 | CONCLUSIONS

For all three different planting systems, the FA of alfalfa in monoculture or in alfalfa–grass mixtures decreased in 2018 (2 yr after management was applied in 2016–2017) compared with 2017. Based on our findings, it is suggested that in order to avoid a decrease in FA, other management strategies should be used, such as herbicide to control weeds, seed planting density, row spacing between species, and potentially increased fertilization levels, especially for mixtures with bermudagrass, considering its high N requirements. Alfalfa stem density decreased with frequent harvests for the A and AB treatments but it remained unchanged during the growing season under the ATF treatment. Therefore, longer harvest intervals can help increase the persistence of alfalfa in the field, especially in monoculture or mixed with bermudagrass. If alfalfa is planted as a mixture with tall fescue, persistence may sharply decline, independent of the harvest management. The nutritive value of these forages is highly correlated with the presence of alfalfa based on stem density, as well as harvest intervals. Although our results disagree with the general knowledge of decreased nutritive value with maturity, we believe that the presence of white clover interfered with the results, as well as the stem density of alfalfa. In Tennessee, if alfalfa is being considered as an option to increase the nutritive value and forage mass of grass pastures, harvest intervals of 42 days are recommended for increased alfalfa persistence.

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CONFLICT OF INTEREST

The authors of the manuscript do not have any conflicts of interest with any organizations or individuals.

ORCID

Renata L. G. Nave
https://orcid.org/0000-0002-6872-8079

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