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# Clover frost-seeding rate effects on productivity and nutritive value of tall fescue pastures during the year of establishment

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**Abstract**

Frost seeding is a low-cost effective strategy to introduce legumes into existing perennial pastures; however, it has been deemed unreliable in the upper southeast USA. We evaluated frost seeding planting rate effects of clover (*Trifolium* spp.) during the year of establishment on productivity and nutritive value of clover-tall fescue [*Festuca arundinacea* (Schreb.) Darbysh.] pastures. Ball (*T. nigrescens* Viv.), crimson (*T. incarnatum* L.), red (*T. pratense* L.), and white (*T. repens* L.) clover were frost seeded in the Piedmont and Coastal Plain for 3 yr (2017–2019). Planting rates were 0*X*, 0.5*X*, 1*X*, 1.5*X*, and 2*X* of the recommended rate (*X*) of 5.6, 13.5, 11.2, and 5.6 kg ha$^{-1}$ for ball, crimson, red, and white clover, respectively. At Coastal Plain, clover accounted for ≤16%. At Piedmont, ball, crimson, red, and white clover accounted for up to 27, 48, 21, and 15%, respectively. Notwithstanding similar clover frequency early in the growing season at both locations, competition from tall fescue in a higher rainfall environment is attributed to the lower clover contribution at Coastal Plain. Increasing clover frost-seeding rate resulted in greater crude protein (130–154 g kg$^{-1}$) and total digestible nutrients (586–610 g kg$^{-1}$) only at Piedmont. The results from this study support the practice of frost-seeding ball, crimson, red, and white clover at planting rates of 5.6, 20.2, 11.2, and 5.6 kg ha$^{-1}$, respectively, into tall fescue pastures in the Piedmont; however, minimal or no benefits of frost seeding clover were observed in the Coastal Plain.

## 1 | INTRODUCTION

Incorporating clover (*Trifolium* spp.) legumes into tall fescue [*Festuca arundinacea* (Schreb.) Darbysh.] pastures has a long history of improving livestock and pasture performance in the U.S. transition zone—the area between the temperate northeast and subtropical southeast (Blaser et al., 1956; McMurphy et al., 1990; Thompson et al., 1993). However, establishment of clover into existing pastures is challenging because of competition from the existing sward and interactions with defoliation management and weather fluctuations (Mueller & Chamblee, 1984; Schlueter & Tracy, 2012).

Frost seeding is the practice of broadcasting clover seed in winter so that freeze–thaw cycles of the soil surface, coupled with later rainfall, provide adequate soil–seed contact (Hall et al., 2020). Particularly in the temperate northeast United States, frost seeding is a recognized low-cost and effective method to increase plant diversity into warm- and cool-season pastures (Gettle et al., 1996; Casler et al., 1999); however, in the U.S. upper southeast, frost seeding has generally been deemed unreliable, and the specific reasons for failure are

**Abbreviations:** ADF, acid detergent fiber; CP, crude protein; NIRS, near-infrared spectroscopy; TDN, total digestible nutrient; *X*, reference planting rate.

unclear (Schlueter & Tracy, 2012); hence, there is still limited adoption of frost seeding in this region.

Previous work with frost-seeded clover into perennial tall fescue pastures in North Carolina and Virginia indicated that control of competition from the grass component, both at planting and during the establishment phase, was more critical than seeding method (no-till vs. broadcasting) for successful establishment of the clover (Mueller & Chamblee, 1984; Schlueter & Tracy, 2012). To the best of our knowledge to date, there are no reports in the literature that have evaluated seeding rates for frost seeding clover into tall fescue in the U.S. southeast. In addition, current available seed of clover in the market is most likely coated, with coatings accounting from 30–50% on a weight basis in our study, which creates another variable when seeding rates are recommended based on bulk weight of seed. Therefore, the objective of this study was to evaluate the effect of five planting rates of four clover species on productivity and nutritive value during the year of establishment when clover were frost seeded into established tall fescue pastures in the Piedmont and Coastal Plain of North Carolina.

**Core Ideas**

- Frost-seeded clover increased diversity and nutritive value of tall fescue pastures at Piedmont.
- At Piedmont, ball, crimson, red, and white clover accounted for 13–48% of the forage.
- Minimal, or no benefits, of frost seeding clover were measured at Coastal Plain.
- At Coastal Plain, clover accounted for ≤16% of the total herbage harvested.

## 2 | MATERIAL AND METHODS

### 2.1 | Experimental sites and preparation

The experimental sites were located at the Lake Wheeler Research Farm at Piedmont (Raleigh, NC; 35.7796° N, 78.6382° W; 97 m asl) and the Cherry Research Station at the Coastal Plain (Goldsboro, NC; 35.3849° N, 77.9928° W; 22 m asl). Selected areas at each site had ≥5-yr-old monoculture tall fescue pastures of ‘Max-Q’ that were mainly used for hay production, received maintenance fertilizer rates, and were sporadically grazed during the fall by sheep at Piedmont and beef at Coastal Plain. A new area was used every year for evaluation of treatments. The soil series were Appling fine sandy loam (fine, kaolinitic, thermic, Typic Kanhapludults) for all sites at Piedmont. At Coastal Plain, soils for the two sites were Leaf silt-loam (fine, mixed, active, thermic Typic Albaquults) and Wagram loamy sand (loamy, kaolinitic, thermic Arenic Kandiudults) for one site.

Oil pH and Mehlich-3 extractable P, K, Ca, Mg, S, and Mn values for all experimental areas are shown in Table 1. Based on the recommendations to grow clover–grass mixtures in North Carolina from the North Carolina Department of Agriculture and Consumer Services Agronomic Services laboratory, the Raleigh 2017 location received 2.5 Mg ha$^{-1}$ of lime in January 2017 and 85 and 130 kg ha$^{-1}$ of P and K, respectively. The Raleigh 2018 location received 62 kg ha$^{-1}$ of K; the Raleigh 2019 location received 5 and 81 kg ha$^{-1}$ of P and K, respectively. The Goldsboro 2018 location received 20 kg ha$^{-1}$ of K. Nitrogen fertilization was banned at each location since the growing season of the year prior to the year when frost-seeding treatments were imposed.

The herbicide Weedar 64 (2,4-dichlorophenoxyacetic acid, dimenthylamine salt; Nufarm, Inc.) was applied on 29 Sept. 2017 and 28 Nov. 2017 at a rate of 0.19 L ha$^{-1}$ to control weeds and in preparation for the Goldsboro 2018 site. The herbicide Weedmaster [Dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid) + Dimethylamine salt of 1,4-dichlorophenoxyacetic acid; Nufarm, Inc.] was applied at a rate of 0.47 L ha$^{-1}$ on 18 Aug. 2017 at the Raleigh 2018 location and on 30 Aug. 2018 at the Raleigh 2019 location. Removing residual grass biomass is critical to encourage clover seedling emergence when frost seeding (Mueller & Chamblee, 1984; Schlueter &Tracy, 2012); thus, the experimental areas were mowed to 7-cm stubble height, and the clipped material was raked out of the plots the day before the frost-seed planting dates (Table 2).

### 2.2 | Treatments and experimental design

The treatments were five planting rates on a pure-live-seed basis for each of four clover species. The clover species were ball (*T. nigrescens* Viv. ‘Grazer’s Select’), crimson (*T. incarnatum* L. ‘Dixie’), red (*T. pratense* L. cultivar SS-0303 RCG), and white (*T. repens* L. ‘Will Ladino’). The five planting rate treatments were: 0*X*, 0.5*X*, 1*X*, 1.5*X*, and 2*X* of the recommended planting rate (*X*) for each clover type based on the literature (Leep, 1989; Martinson & Shaeffer, 2020; Mueller & Chamblee, 1984; Schlueter & Tracy, 2012; NRCS, 2009). Hence, the reference planting rate values (*X*) were 5.6, 13.5, 11.2, and 5.6 pure live seed kg ha$^{-1}$ for ball, crimson, red, and white clover, respectively. Clover seeds were coated for all clover, and coatings ranged from 34 to 50% on a weight basis based on the seed bag labels. Treatments were allocated to experimental units arranged in a randomized complete block design replicated three times. There was one trial per clover type; hence, there were 15 experimental units total per clover type (five planting rates × three

**TABLE 1** Soil chemical analysis from samples taken at the initiation of the study

| Region | Environment | Sampling date | pH | P | K | Ca | Mg | S | Mn |
|---|---|---|---|---|---|---|---|---|---|
| | | | | mg kg$^{-1}$ | | | | | |
| Coastal Plain | Goldsboro 2017 | Jan. 2017 | 7.0 | 266 | 229 | 134 | 34 | 19 | 12 |
| | Goldsboro 2018 | Nov. 2017 | 6.8 | 77 | 199 | 112 | 30 | 53 | 20 |
| | Goldsboro 2019 | Jan. 2019 | 6.2 | 311 | 184 | 90 | 30 | 13 | 79 |
| Piedmont | Raleigh 2017 | Dec. 2016 | 5.6 | 98 | 45 | 96 | 18 | 10 | 18 |
| | Raleigh 2018 | Nov. 2017 | 6.3 | 179 | 149 | 104 | 32 | 26 | 32 |
| | Raleigh 2019 | Nov. 2018 | 6.3 | 136 | 92 | 108 | 35 | 20 | 21 |

**TABLE 2** Dates for planting, maintenance grass control clipping, clover frequency sampling, and herbage mass sampling

| Region | Environment | Planting date | Grass control clipping | Clover frequency | Herbage mass |
|---|---|---|---|---|---|
| Coastal Plain | Goldsboro 2017 | 2 Feb. | 6 Jan., 7 Apr. | 29 Mar. | 13 Jun. |
| | Goldsboro 2018 | 2 Feb. | 25 Jan., 19 Apr. | 2 Mar. | 6 Jun. |
| | Goldsboro 2019 | 14 Feb. | 1 Feb., 17 Apr. | 8 Apr. | 20 May. |
| Piedmont | Raleigh 2017 | 13 Feb. | 19 Jan., 19 Apr. | 14 Apr. | 1 Jun. |
| | Raleigh 2018 | 8 Feb. | 1 Feb., 3 May. | 6 Apr. | 1 Jun. |
| | Raleigh 2019 | 18 Feb. | 18 Jan., 10 Apr. | 23 Apr. | 14 May. |

replicates). The experimental unit size was 5-m long by 3-m wide with 1-m alleys.

## 2.3 | Establishment and plot management

Weighed seed for each treatment was mixed with ∼0.5 kg of sand to help improve seed dispersion when broadcasting. The seed–sand mix was hand broadcasted by walking the plots at least two times in perpendicular directions. Specific dates of frost-seed planting and harvesting events, as well as clipping events to control competition from tall fescue, are listed in Table 2. Controlling grass competition is critical for establishment of frost-seeded white clover in tall fescue pastures (Mueller & Chamblee, 1984; Tracy & Schlueter, 2012); therefore, following frost-seed planting of the clover, frequent mowing with a rotary mower set to ≥10-cm height was used to control competition from tall fescue and to allow sunlight penetration to encourage establishment of the young clover seedlings. Observed monthly rainfall and monthly average maximum and minimum temperatures for the experimental period (January to June) for Piedmont and Coastal Plain are presented in Figure 1.

## 2.4 | Response variables

### 2.4.1 | Clover frequency

Frequency of occurrence of clover is a measurement of the relative distribution of emerged clover in the sampling area (Castillo et al., 2013). Clover frequency was determined when clover emergence was observed in every plot, which occurred between 53 and 64 d after planting (Table 2) except for Goldsboro 2019, which occurred 28 d after planting. The sampling dates for clover frequency occurred between the two clipping events for grass control (Table 2) except for Piedmont 2019, which occurred after the second grass-control clipping event. A 1-m$^2$ (1 by 1 m) quadrat was randomly placed in two separate sampling points of each experimental unit. The quadrat was divided into 25 20- by 20-cm squares (five rows of five). Presence or absence of clover was determined in all 25 squares per quadrat. Clover frequency was calculated as the percentage of the total number of cells assessed in each quadrat where clover was present. The frequency values were averaged between the two quadrat locations to obtain an overall clover frequency per experimental unit.

### 2.4.2 | Herbage mass and botanical composition

Herbage mass samples were collected by clipping a 2.7-m$^{-2}$ area (3-m long by 0.9-m wide) to 7-cm stubble height using a walk-behind sickle bar mower. The harvested forage was weighed fresh in the field, then two grab-samples (∼0.5–1 kg) were collected. One sample was weighed fresh in the field and subsequently dried at 60 ˚C until constant weight to determine dry matter concentration to calculate herbage mass and to determine nutritive value. The second sample was used to determine botanical composition by weight. Botanical

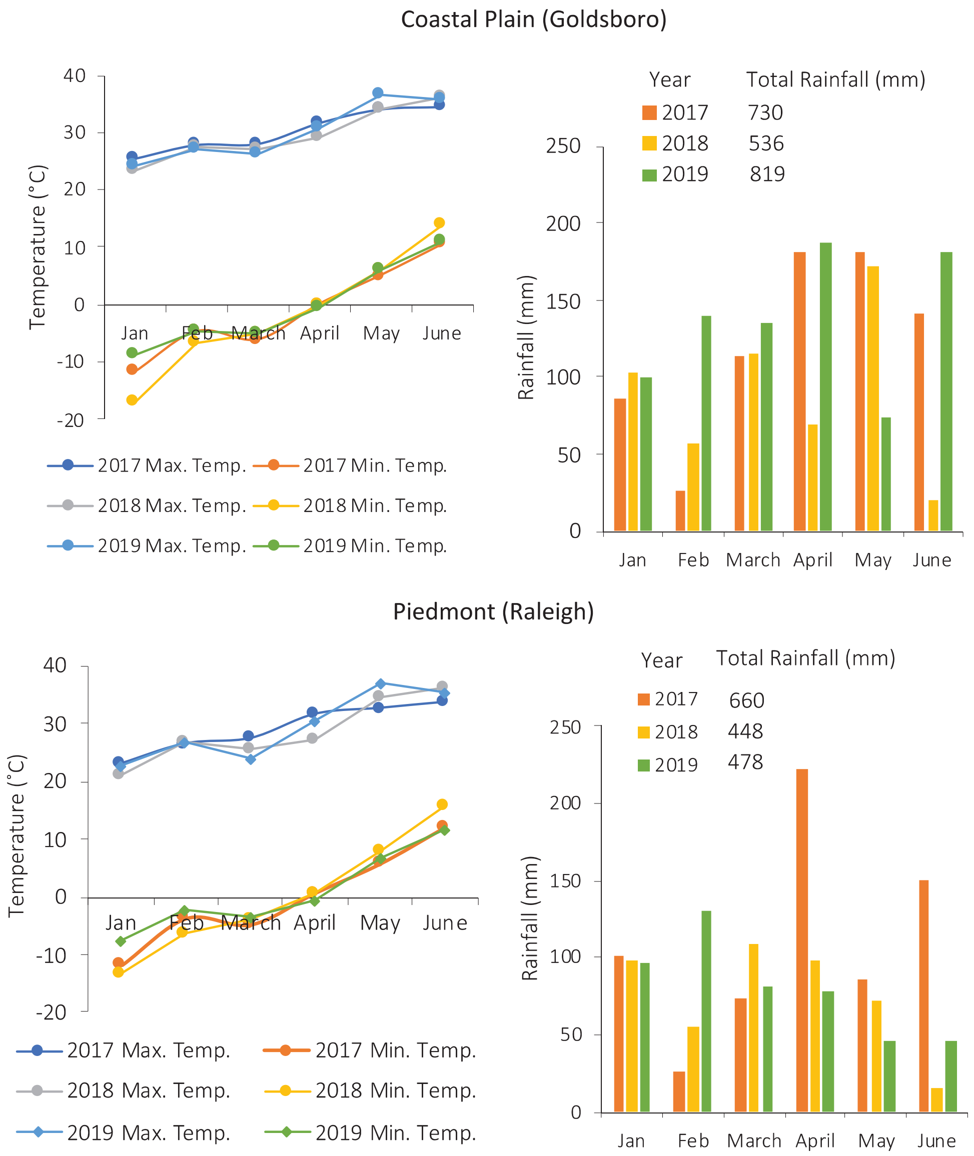


**FIGURE 1** Observed monthly rainfall and maximum and minimum temperatures during the experimental period at the Coastal Plain and Piedmont locations. Total rainfall values in figures correspond to the January to June experimental period.

composition was determined by hand separating the fresh forage in three components: tall fescue, clover, and others. Then, the separated samples were dried at 60 ˚C until constant weight, the dry weights for each component were recorded, and the percentage by weight of each component was determined for each experimental unit. Samples for determination of herbage mass and botanical composition values were collected once during the late-May to early June timeframe, and exact dates of the sampling events are provided in Table 2.

### 2.4.3 | Nutritive value

Dried samples were ground using a Wiley mill (A. H. Thomas) to <1-mm particle size in preparation for analysis of crude protein (CP) and acid detergent fiber (ADF). Concentration of CP and ADF were determined using near-infrared spectroscopy (NIRS) models developed for this experiment. Samples were scanned with a Foss NIRS Model 6500 device (Foss North America) and NIRS model development was performed using a data analysis pipeline written in R environment (R Core Team, 2016). To obtain a calibration for CP and ADF, a total of 157 samples (~52% of the total number of samples in the dataset) were selected based on their spectral data that represented the population of samples collected in this trial. The selected samples were sent to a commercial laboratory for wet chemistry analysis of CP and ADF concentrations. In summary from the laboratory analytical procedures manual (Dairy One, 2015), concentration of CP was calculated by multiplying the concentration of total N (determined by dry combustion using a LECO CN628) by 6.25. The ADF concentration was determined using Method 12 of the ANKOM Fiber Analyzer (ANKOM Technology). Fit statistics of NIRS models for CP were 0.94 and 0.5 for $r^2$ and standard error of calibration, respectively, and 0.91 and 0.6 for $r^2$ standard error of cross-validation, respectively. For ADF, 0.95 and 0.3 for $r^2$ and standard error of calibration, respectively, and 0.89 and 0.5 $r^2$ and standard error of cross-validation, respectively. The ADF values were used to calculate total digestible nutrient (TDN) concentration following the equation for 'mixed forage' used by the North Carolina Department of Agriculture and Consumer Services Feed and Forage Laboratory [TDN = 87.8364 − (0.69561 × ADF)]. Estimates of TDN are the preferred method to balance the energy requirements of forage-based rations for beef cattle in North Carolina (Freeman et al., 2016; Poore, 2014; Kunkle et al., 2000).

### 2.5 | Statistical analyses

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, 2010). Because the objective was to evaluate the effect of frost-seeding planting rates for each clover, and not to compare among clover, analyses were performed by clover type within each region. Statistical models were set up per clover type. For all models, the seeding rate was modeled as a fixed effect and year and block within each year as random effects. When a significant seeding rate effect was declared, polynomial contrasts (linear, quadratic, and cubic) were used to determine the nature of responses to seeding rate effect. In addition, individual treatment mean responses were separated using the LINES option of GLIMMIX in SAS. All means reported in the text are least square means. Plots of model residuals were used to check for normality. Treatments effects were considered different if $P \leq .05$.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Clover frequency

At Coastal Plain, clover frequency increased (linear, quadratic, and cubic effects for all clover except crimson clover, which did not have a cubic effect) from 0 to 75, 74, 87, and 70% for ball, crimson, red, and white clover, respectively, as clover frost-seeding planting rate increased from 0$X$ to 2$X$ (Table 3). By examining individual treatment-mean separation, the greatest clover frequency values were first achieved when planting rates were 8.4 kg ha$^{-1}$ for ball clover (1.5$X$ planting rate and 68% frequency), 13.5 kg ha$^{-1}$ for crimson ($X$ planting rate and 67% frequency), 11.2 kg ha$^{-1}$ for red clover ($X$ planting rate and 77% frequency), and 8.4 kg ha$^{-1}$ for white clover (1.5$X$ planting rate and 63% frequency).

At Piedmont, clover frequency increased from 0 to 77, 86, 73, and 70% for ball, crimson, red, and white clover, respectively (linear, quadratic, and cubic effects for all clover except ball clover, which did not have a cubic effect) (Table 3). Further analysis to separate individual treatment-mean responses indicated that greatest clover frequency values were first achieved at clover frost-seeding planting rates of 5.6 kg ha$^{-1}$ for ball clover ($X$ planting rate and 69% frequency), 20.3 kg ha$^{-1}$ for crimson (1.5$X$ planting rate and 77% frequency), 11.2 kg ha$^{-1}$ for red clover ($X$ planting rate and 75% frequency), and 5.6 kg ha$^{-1}$ for white clover ($X$ planting rate and 59% frequency).

Averaged across treatments, clover frequency values at Coastal Plain were 49, 52, 63, and 48% for ball, crimson, red, and white clover, respectively. At Piedmont, averaged across treatments, clover frequency values were 56, 55, 55, and 49% for ball clover, crimson, red, and white clover, respectively. Although clover frequency values were, in general, similar between regions for all clover species (≤8% points difference between Coastal Plain and Piedmont), ball and white clover required 1.5$X$ planting rate at Coastal Plain, compared with the $X$ planting rate at Piedmont, to achieve similar results. This response probably is due to the less-than-optimal conditions for establishment of ball and white clover at Coastal Plain. Ball and white clover have smaller seed sizes and greater number of seeds per kilogram of bulk seed compared with red and crimson clover, and reports in the literature indicate that ball and white clover are best adapted to loam and clay soils (Ogle, 2008; Smith et al., 2006), like the soils at Piedmont, and less adapted to sandy-type soils like those at Coastal Plain. Crimson and red clover are more broadly adapted; however, it is not completely clear why the planting rate to achieve greatest clover frequency for crimson clover was greater at Piedmont (1.5$X$ planting rate) than at Coastal Plain ($X$ planting rate). The greatest canopy cover for red clover was consistently achieved at the planting rate of 11.2 kg ha$^{-1}$ ($X$ planting rate) at both regions.

### 3.2 | Herbage mass and botanical composition

At Coastal Plain, total herbage mass values averaged across planting rates were 1,144; 1,105; 1,315; and 1,184 kg ha$^{-1}$ for plots frost seeded with ball, crimson, red, and white clover, respectively. The effect of increasing clover frost-seeding rates on herbage mass was significant only for red clover. Increasing red clover frost-seeding planting rate from 0 to 22.4 kg ha$^{-1}$ increased total herbage mass (linear effect) from 930 to 1,621 kg ha$^{-1}$ (Table 4). Although there were significant planting rate effects on the percentage clover for red and white

**TABLE 3** Frequency of clover (*Trifolium* spp.) [$X$ = 5.6, 13.5, 11.2, and 5.6 kg ha$^{-1}$ for ball (*T. nigrescens* Viv.), crimson (*T. incarnatum* L.), red (*T. pratense* L.), and white (*T. repens* L.) clover, respectively] in the Coastal Plain and Piedmont of North Carolina. Data represent three year–location combinations and three replicates per year–location

| Region | Planting rate | Ball | Crimson | Red | White |
|---|---|---|---|---|---|
| | | % | | | |
| Coastal Plain | 0X | 0 | 0 | 0 | 0 |
| | 0.5X | 44 | 45 | 68 | 52 |
| | X | 56 | 67 | 77 | 54 |
| | 1.5X | 68 | 74 | 82 | 63 |
| | 2X | 75 | 74 | 87 | 70 |
| | Polynomial contrast[a] | L, Q, C | L, Q | L, Q, C | L, Q, C |
| Piedmont | 0X | 0 | 0 | 0 | 0 |
| | 0.5X | 55 | 53 | 54 | 50 |
| | X | 69 | 59 | 75 | 59 |
| | 1.5X | 81 | 77 | 74 | 66 |
| | 2X | 77 | 86 | 73 | 70 |
| | Polynomial contrast | L, Q | L, Q, C | L, Q, C | L, Q, C |

*Note*. C, cubic; L, linear; Q, quadratic.

clover, overall, clover accounted for ≤16% of the harvested herbage. The grass component constituted ≥79% of the harvested herbage and weed infestation ≤12%.

At Piedmont, total herbage mass values averaged across clover planting rates were 907, 885, 887, and 773 kg ha$^{-1}$ for ball, crimson, red, and white clover, respectively. Consistent with the response measured for red clover at Coastal Plain, increasing red clover planting rate from 0 to 22.4 kg ha$^{-1}$ increased total herbage mass (linear and cubic effects) from 693 to 1,176 kg ha$^{-1}$ (Table 4). Increasing clover planting rates from 0*X* to 2*X* increased the percentage of clover in the harvested herbage for all clover species; specifically, from 0 to 27% for ball (linear and quadratic effect), to 48% (linear effect) for crimson, to 21% for red (linear and quadratic effect), and to 13% for white clover (linear effect) (Table 4). In general, as the amount of clover in the harvested herbage increased, both the grass and weed components decreased. Across treatments, the grass component ranged from 49 to 89% and the weed component from 3 to 19% of the total harvested herbage.

Maximum and minimum monthly temperatures were similar between Coastal Plain and Piedmont (Figure 1); however, total rainfall during the experimental period was consistently greater at Coastal Plain by 70, 88, and 342 mm in 2017, 2018, and 2019, respectively. The dates for planting and grass-control clippings occurred earlier, and sampling dates later, by approximately 1–2 wk at Coastal Plain vs. Piedmont (Table 2). Despite similar clover frequency values at both locations when measured between grass-control clipping events (Table 3), the amount of clover in the harvested herbage at Coastal Plain was ≤16% across treatments compared with 15–48% across treatments at Piedmont (Table 4). At Coastal Plain, tall fescue productivity for the 0*X* treatment was 930 kg ha$^{-1}$ for the 0*X* treatment; at Piedmont it was 693 kg ha$^{-1}$ (Table 4); hence, it is possible that the existing tall fescue outcompeted the establishing clover seedling by means of faster tall fescue spring regrowth coupled with higher rainfall at Coastal Plain. Continuous measurements of soil moisture, as well as measurement of the dynamics of the light environment experienced by clover during the establishment phase, could improve our understanding about the underlying causes of unreliable clover frost seeding and the observed lower clover contribution in the harvested herbage in the Coastal Plain notwithstanding the high clover frequency measured early in the season.

Frost seeding Will Ladino white clover at a rate of 5.6 kg ha$^{-1}$ into existing tall fescue pastures was previously reported as a successful strategy to achieve a grass–legume sward in the Piedmont of North Carolina by Mueller and Chamblee (1984). The authors reported that percentage clover accounted for 23% in the first harvest of the year of establishment, and from 38 to 81% of the total seasonal harvested herbage across 4 yr (Mueller & Chamblee, 1984). In contrast, the results of our study across planting rates in the Piedmont indicate that white clover accounted for up to 15% in the first harvest of the year of establishment compared with up to 23% for red, 48% for crimson, and 35% for ball clover (Table 4). Schlueter and Tracy (2012) broadcast overseeded a clover seed mix (seeding rates of 2.2 kg ha$^{-1}$ of white clover 'Kopu II' plus 2.2 kg ha$^{-1}$ of Will Ladino white clover plus 4.4 kg ha$^{-1}$ of 'Liberty' red clover) into a dominantly tall fescue–Kentucky bluegrass (*Poa pratensis* L.) pasture with a smaller proportion of orchardgrass (*Dactylis glomerata* L.). The authors reported that the clover mix accounted for ≤27% of the total harvested herbage for the June harvest (first harvest of the season) across

**TABLE 4** Herbage mass (HM) and botanical composition as a function of pure live seed frost-seeding planting rate for four clover (*Trifolium* spp.) [$X$ = 5.6, 13.5, 11.2 and 5.6 kg ha$^{-1}$ for ball (*T. nigrescens* Viv.), crimson (*T. incarnatum* L.), red (*T. pratense* L.), and white (*T. repens* L.) clover, respectively] in the Coastal Plain and Piedmont of North Carolina. Data represent three year–location combinations and three replicates per year–location

| | | Ball | | | | Crimson | | | | Red | | | | White | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Region** | **Planting rate** | **HM** | **Grass** | **Legume** | **Other** | **HM** | **Grass** | **Legume** | **Other** | **HM** | **Grass** | **Legume** | **Other** | **HM** | **Grass** | **Legume** | **Other** |
| | | kg ha$^{-1}$ | % | | | kg ha$^{-1}$ | % | | | kg ha$^{-1}$ | % | | | kg ha$^{-1}$ | % | | |
| Coastal Plain | 0*X* | 930 | 96 | 0 | 4 | 930 | 96 | 0 | 4 | 930 | 96 | 0 | 4 | 930 | 96 | 0 | 4 |
| | 0.5*X* | 1013 | 85 | 6 | 8 | 981 | 86 | 10 | 4 | 1318 | 82 | 16 | 3 | 1256 | 88 | 9 | 3 |
| | *X* | 1329 | 85 | 4 | 12 | 1141 | 80 | 10 | 10 | 1301 | 84 | 11 | 5 | 1262 | 84 | 16 | 1 |
| | 1.5*X* | 1212 | 89 | 7 | 4 | 1326 | 79 | 15 | 6 | 1403 | 83 | 15 | 2 | 1216 | 86 | 14 | 0 |
| | 2*X* | 1234 | 88 | 10 | 3 | 1146 | 85 | 13 | 1 | 1621 | 82 | 14 | 4 | 1256 | 85 | 15 | 0 |
| | Polynomial contrast[a] | NS | NS | NS | NS | NS | L, Q | NS | NS | L | L, Q | L, Q | NS | NS | NS | L, Q | NS |
| Piedmont | 0*X* | 693 | 81 | 0 | 19 | 693 | 81 | 0 | 19 | 693 | 82 | 0 | 18 | 693 | 81 | 0 | 19 |
| | 0.5*X* | 899 | 77 | 12 | 11 | 821 | 67 | 24 | 9 | 928 | 89 | 5 | 6 | 756 | 85 | 7 | 8 |
| | *X* | 939 | 63 | 27 | 10 | 937 | 73 | 24 | 3 | 966 | 65 | 23 | 12 | 802 | 85 | 11 | 4 |
| | 1.5*X* | 940 | 61 | 35 | 4 | 1034 | 61 | 35 | 4 | 673 | 76 | 16 | 8 | 764 | 78 | 15 | 7 |
| | 2*X* | 1065 | 67 | 27 | 6 | 941 | 49 | 48 | 3 | 1176 | 73 | 21 | 6 | 850 | 83 | 13 | 4 |
| | Polynomial contrast | NS | NS | L, Q | NS | NS | L | L | L | L, C | L | L, Q | NS | NS | NS | L | L |

*Note*. C, cubic; L, linear; NS, not significant; Q, quadratic.

**TABLE 5** Crude protein (CP) and total digestible nutrient (TDN) concentrations of the harvested herbage (grass + legume) as a function of pure live seed frost-seeding planting rate for four clover (*Trifolium* spp.) [$X = 5.6, 13.5, 11.2$, and 5.6 kg ha$^{-1}$ for ball (*T. nigrescens* Viv.), crimson (*T. incarnatum* L.), red (*T. pratense* L.), and white (*T. repens* L.) clover, respectively] in the Coastal Plain and Piedmont of North Carolina. Data represent of three year–location combinations and three replicates per year-location

| Region | Planting rate | Ball | | Crimson | | Red | | White | |
|---|---|---|---|---|---|---|---|---|---|
| | | CP | TDN | CP | TDN | CP | TDN | CP | TDN |
| | | g kg$^{-1}$ | | | | | | | |
| Coastal Plain | 0*X* | 120 | 594 | 120 | 594 | 120 | 594 | 120 | 594 |
| | 0.5*X* | 122 | 589 | 117 | 591 | 126 | 596 | 122 | 590 |
| | *X* | 123 | 595 | 126 | 594 | 125 | 596 | 131 | 599 |
| | 1.5*X* | 123 | 591 | 117 | 590 | 132 | 597 | 133 | 595 |
| | 2*X* | 119 | 591 | 122 | 593 | 134 | 604 | 129 | 594 |
| | Polynomial contrast | NS | NS | NS | NS | NS | NS | NS | NS |
| Piedmont | 0*X* | 134 | 589 | 132 | 586 | 131 | 586 | 130 | 586 |
| | 0.5*X* | 132 | 596 | 132 | 594 | 124 | 586 | 132 | 595 |
| | *X* | 141 | 604 | 135 | 596 | 141 | 594 | 132 | 590 |
| | 1.5*X* | 150 | 611 | 145 | 599 | 140 | 591 | 137 | 593 |
| | 2*X* | 154 | 610 | 149 | 604 | 143 | 598 | 137 | 595 |
| | Polynomial contrast | L | L | L | L | L, C | L | NS | NS |

*Note*. C, cubic; L, linear; NS, not significant; Q, quadratic.

years and concluded that studies combining weather variables and pasture management could improve our understanding of what makes successful clover establishment possible.

## 3.3 | Nutritive value

There was no effect of clover frost-seeding rates on CP and TDN at Coastal Plain (Table 5). Averaged across frost-seeding rates, the concentrations of CP and TDN were 121 and 592 g kg$^{-1}$ for ball, 121 and 592 g kg$^{-1}$ for crimson, 128 and 597 g kg$^{-1}$ for red, and 127 and 594 g kg$^{-1}$ for white clover, respectively. At Piedmont, CP and TDN values increased as a function of increasing clover frost-seeding rate for all clover except for white clover (Table 5). Increasing frost-seeding rates from 0*X* to 2*X* increased CP and TDN concentration values at Piedmont from 134 to 154 (linear effect) and 589 to 610 g kg$^{-1}$ (linear effect) for ball, from 132 to 149 (linear effect) and from 586 to 604 g kg$^{-1}$ (linear effect) for crimson, from 131 to 143 (linear and cubic effects) and from 586 to 598 g kg$^{-1}$ (linear effect) for red clover, respectively. For white clover, concentrations averaged across seeding rates were 134 g kg$^{-1}$ for CP and 592 g kg$^{-1}$ for TDN.

Clover percentage, determined as botanical composition by weight from the total herbage harvested, was positively correlated with CP and TDN at Piedmont and only with CP at Coastal Plain (Table 6). Although the presence of clover resulted in greater CP and TDN values mainly at Piedmont, the grass component alone (0*X* planting rate) had CP concentration values on average of 120 g kg$^{-1}$ at Coastal Plain and

**TABLE 6** Coefficients of linear correlation (*r*) between percentage clover determined on a botanical composition by weight basis and concentrations of crude protein (CP) and total digestible nutrients (TDN) of the total harvested herbage mass. Data used in this analysis ($n = 20$) were the average of four replicates and 2 yr for four clover species (*Trifolium nigrescens*, *T. incarnatum*, T. *pratense*, *T. repens*) planted at five frost-seeding rates ($n = 20$)

| Region | Item, range (min–max) | CP | TDN |
|---|---|---|---|
| | | g kg$^{-1}$ | |
| Coastal Plain | Percentage clover (0–16) | (117–134) | (589–604) |
| | | .56*** | .28 NS |
| Piedmont | Percentage clover (0–48) | (124–154) | (586–611] |
| | | .78*** | .82*** |

***Significant at the .001 probability level. NS, not significant.

≥130 g kg$^{-1}$ at Piedmont, both values exceeding the recommended CP concentration of ∼105 g kg$^{-1}$ suitable to meet the CP dietary needs of a lactating beef cow in the first 90 d after calving if forage was the only source of feed. Also, average TDN values for the grass component (0*X* planting rate) in both regions met or were barely under the recommended TDN concentration of 600 g kg$^{-1}$ (NRC, 1996). If the tall fescue component of the pasture is wild-endophyte infested tall fescue, then adding clover to achieve a grass–legume mixture is a recognized practice to manage fescue toxicosis. This practice improves animal performance compared to when livestock consumes wild-endophyte infected tall fescue alone (Aiken & Strickland, 2013; Aiken et al., 2016),especially during the spring grazing season with clover presence ≥20%

in the tall fescue–clover mixture (McMurphy et al., 1990; Thomson et al., 1993; Fribourg et al., 1991; Hoveland et al., 1981).

## 4 | SUMMARY AND CONCLUSIONS

Overall, there was no effect of increasing clover frost-seeding rates on total herbage mass except for red clover for which total herbage mass increased as a function of increasing planting rates at Coastal Plain and Piedmont. Increasing clover planting rates consistently resulted in the clover component accounting for a greater percentage of the total herbage harvested, except for ball and crimson clover at the Coastal Plain. Across treatments at Coastal Plain, both red and white clover accounted for up to 16% of the total herbage harvested compared to Piedmont where ball, crimson, red, and white clover accounted for up to 27, 48, 21, and 15%, respectively. Notwithstanding similar clover frequency values when measured between grass-control clipping events, the amount of clover in the harvested herbage at Coastal Plain was lower (≤16% across treatments) than at Piedmont (15–48% across treatments). Greater amount of tall fescue and faster tall fescue spring regrowth, coupled with higher rainfall, are attributed to the lower contribution of frost-seeded clover at Coastal Plain; however, continuous monitoring of soil moisture and light environment experienced by the establishing clover will contribute to a better understanding of the underlying causes of unreliable clover frost seeding at Coastal Plain. There was a positive effect of increasing clover frost-seeding rate on CP and TDN concentration values only at Piedmont; however, compared with the values required to meet the dietary needs of a lactating beef cow in the first 90 d after calving if forage was the only source of feed (i.e.,105 g kg$^{-1}$ for CP and 600 g kg$^{-1}$ for TDN concentration), the CP and TDN concentrations of the grass component alone were higher for CP and barely under for TDN in both regions. Based on the clover frequency values and considering herbage mass and nutritive value responses measured, planting rates of 5.6, 20.2, 11.2, and 5.6 kg ha$^{-1}$ for ball, crimson, red, and white clover are recommended for frost-seeded clover in tall fescue pastures of the Piedmont. Results from this study support the practice of frost seeding ball, crimson, red, and white clover into tall fescue pastures as a strategy to create a grass–legume mixture in the Piedmont; however, minimal or no benefits of frost seeding clover were observed in the Coastal Plain.

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## AUTHOR CONTRIBUTIONS

Miguel S. Castillo: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. Perejitei E. Bekewe: Investigation; Methodology; Project administration; Resources. Raul Rivera: Investigation; Methodology; Project administration; Resources.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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