# Soil-Test Biological Activity with the Flush of CO<sub>2</sub>: IV. Fall-Stockpiled Tall Fescue Yield Response to Applied Nitrogen

Alan J. Franzluebbers,\* Smriti Pehim-Limbu, and Matt H. Poore

#### ABSTRACT

Fall stockpiling of tall fescue (Lolium arundinaceum) in the southeastern United States is promoted as an ecologically favorable cattle management approach to avoid the financial and environmental burdens of winter hay feeding. We hypothesized that soil N mineralization should be an important factor controlling forage yield response to N fertilizer. We conducted 55 N fertilizer trials in combination with analyses of soil C and N fractions at multiple locations in Georgia, North Carolina, Virginia, and West Virginia during two seasons. Plant-available N, as a combination of residual inorganic N + mineralizable N at depth of 0 to 10 cm, was significantly negatively related with extent of forage dry matter response to N fertilizer input. Large variations in economically optimum N fertilizer requirement (EONR) occurred among fields, but when several fields were averaged along a gradient of soil biological activity, a strong negative yield response with increasing soil-test biological activity emerged. With moderate soil-test biological activity of 200 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil 3 d<sup>-1</sup>, EONR was 20 kg N Mg<sup>-1</sup> forage dry matter (a value similar to current N fertilizer recommendations). However, with progressively greater soil-test biological activity up to 600 mg CO<sub>2</sub>–C kg<sup>-1</sup> soil 3 d<sup>-1</sup>, EONR declined in a nonlinear manner to near zero. These results illustrate that N fertilizer recommendations for fall stockpiled tall fescue pastures should be a function of soil-test biological activity as an indicator of biologically active N. Greater economic and environmental sustainability would likely be attainable with a shift to recognizing soil biological activity in an ecologically oriented fertilization paradigm.

#### Core Ideas

- Soil biological activity is a reliable indicator of soil nitrogen availability.
- Nitrogen fertilization of fall stockpiled tall fescue should be adjusted based on soil testing.
- The flush of CO<sub>2</sub> is a robust indicator of soil biological activity.
- A new paradigm of soil testing based on soil biological activity is possible.

Copyright © 2018 by the American Society of Agronomy 5585 Guilford Road, Madison, WI 53711 USA This is an open access article distributed under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

ALL FESCUE [Lolium arundinaceum (Schreb.) Darbysh. = Schedonorus arundinaceus (Schreb.) Dumort.] is the most important perennial, cool-season forage in the southeastern United States, distributed on ~14 Mha of land in the United States (Buckner et al., 1979). Tall fescue is persistent under extreme summer heat conditions in the region, generally palatable, of high nutritive value to livestock, and able to withstand a variety of stocking densities and strategies imposed over time. Of great concern with naturalized stands of tall fescue is the production of ergot alkaloids by the endophytic fungus [Neotyphodium coenophialum (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin]. Common-seed plantings and old, naturalized stands of tall fescue harbor this wild-type endophyte that causes mild to extreme cases of animal health disorders when forage and seed-heads are consumed (Stuedemann and Hoveland, 1988). However, the endophyte is also a key reason for its tolerance to grazing pressure and harsh environmental conditions, particularly heat and drought. Therefore, a variety of reasons are available why farmers and agricultural advisors have mixed feelings about the merits of tall fescue.

One strategy to limit exposure of grazing livestock to the deleterious effects of ergot alkaloid consumption is to accumulate forage in the fall for winter grazing (i.e., fall stockpile). Fall stockpiling of tall fescue is promoted as an ecologically favorable cattle management approach to avoid the financial and environmental burdens of winter hay feeding (Poore and Drewnoski, 2010). Ergot alkaloid concentration is highest in spring and fall growth periods (Belesky et al., 1988; Rottinghaus et al., 1991). Deferring grazing in the fall to the winter allows herbage mass to accumulate to a sufficient level and allows freezing, early winter-time temperatures to partially desiccate the forage and reduce the concentration of ergot alkaloids (Kallenbach et al., 2003). Therefore, farmers are interested in optimizing fall stockpile growth to increase overall farm efficiency and avoid deleterious animal gain situations with wild-type endophyte infection of tall fescue.

Tall fescue dry matter (DM) production can be increased substantially with N fertilizer inputs, whether spring or fall applied and when harvested throughout the year (Fribourg and Bell, 1984; Wolf and Opitz von Boberfeld, 2003). The typical DM increase with N fertilizer input has been summarized

Abbreviations: CV, coefficient of variation; DM, dry matter; EONR, economically optimum nitrogen fertilizer requirement; TOC, total organic carbon; TSN, total soil nitrogen.

Published in Agron. J. 110:2033–2049 (2018) doi:10.2134/agronj2018.03.0146 Available freely online through the author-supported open access option

A.J. Franzluebbers, USDA–Agricultural Research Service, 3218 Williams Hall, NCSU Campus Box 7620, Raleigh NC 27695; S.Pehim-Limbu, North Carolina State Univ., Dep. Crop and Soil Sciences, Raleigh NC 27695; M. H. Poore, North Carolina State Univ., Dep. Animal Science, Raleigh NC 27695. Received 2 Mar. 2018. Accepted 5 June 2018. \*Corresponding author (Alan.Franzluebbers@ars.usda.gov).

across a number of studies as 7 to 33 kg forage DM kg $^{-1}$  N input (Poore and Drewnoski, 2010).

Current N fertilizer recommendations for tall fescue have similarities and differences among states in the eastern United States. In North Carolina, the N fertilizer recommendation for tall fescue hay or baleage is 112 to 224 kg N ha<sup>-1</sup> yr<sup>-1</sup> in split application after each cutting (Castillo et al., 2016). The recommendation can be reduced to 75% of the standard rate if continuously grazed and to 50% of the standard rate if grazed rotationally (due to return of feces to the pasture). Recommendation guidelines are for the high end of the standard rate range on sites with low fertility index based on high expected yield response (Castillo et al., 2016). Implicitly, this recommendation suggests that soil biological activity should be a key diagnostic feature of N fertilizer recommendation. Nitrogen fertilizer at a rate of 56 to 90 kg N ha<sup>-1</sup> is recommended specifically for fall stockpiled tall fescue in North Carolina (Castillo et al., 2018). In Virginia and Tennessee, the recommended N fertilizer rate is 70 to 90 kg N ha<sup>-1</sup>, independent of management or pasture condition (Johnson and Smith, 2004). In Georgia and Mississippi, the recommended N fertilizer rate for tall fescue is 45 to 70 kg N ha<sup>-1</sup> (Hancock and Josey, 2008; Lemus, 2008). In West Virginia, the recommended N fertilizer rate is 56 to 112 kg N ha<sup>-1</sup> on fall-stockpiled tall fescue (Rayburn, 1993).

Soil testing is not considered when making N fertilizer recommendations in most states in the eastern United States. Soil tests could be used for making recommendations, but those indices that have been proposed take too much time, are laborious, are costly compared with the expected return, or are not sufficiently accurate to be effective. The standard method of determining net N mineralization is through subsequent leaching and incubation (Stanford and Smith, 1972), but it requires 32 wk of incubation for a single sample. Another proposed method is anaerobic incubation for 1 wk (Keeney and Bremner, 1966), but it relies on an anaerobic process that is not commonly encountered in the field. Recently, a simple, rapid, and robust indicator of soil N availability has been proposed through the short-term mineralization of C as it directly relates to longer-term N mineralization (Franzluebbers, 2016). In 47 soils to be used for corn production in North Carolina and Virginia, the flush of CO<sub>2</sub> in 3 d was strongly associated with net N mineralization during 24 d  $(r^2 = 0.77)$  (Franzluebbers et al., 2018). Our goal in this research was to evaluate the effectiveness of this indicator to assess soil N availability and, if useful, to predict N fertilizer requirements to achieve economically optimum production.

Our hypothesis was that soils varying in potential soil biological activity, as determined from short-term C mineralization (also known as the flush of  $CO_2$ ), would lead to differences in how fall-stockpiled tall fescue pastures respond in DM production to N fertilizer application. Fields with high soil-test biological activity were hypothesized to have sufficient mineralizable N and therefore to have a low likelihood of significant yield response to N fertilizer inputs. In contrast, fields with low soil-test biological activity would be assumed to have the more typically expected high yield response to N fertilizer.

### MATERIALS AND METHODS Experimental Setup

A total of 57 field trials in Georgia, North Carolina, Virginia, and West Virginia were evaluated for fall-stockpiled forage yield response to N fertilizer application in 2015 and 2016 (Table 1). At two field trials in 2015, accidental cattle grazing prevented harvest data collection. As a result, 20 fields were evaluated in 2015 and 35 fields in 2016. Fields were selected based on the presence of suitable tall fescue composition, collaborative interest of farmers and research station managers, and a desire to obtain a diversity of conditions within a farm and region. Experimental fields were in three main physiographic regions, including the relatively flat Coastal Plain, the undulating hills of the Piedmont, and the steep slopes and valleys of the Appalachian Mountains (Table 1). Mean annual temperature among sites was  $13.9 \pm 1.8$  °C, and mean annual precipitation was  $1143 \pm$ 112 mm. Seasonal precipitation of relevance to this investigation is reported in Table 1. We considered the September–November period as most relevant for fall growth, and therefore those fields that received <120 mm during this period were considered drought affected. Drought affected 10 of the 55 fields; all drought events occurred in 2016, when an unusually long period without precipitation was experienced throughout northern Georgia, western North Carolina, and western Virginia. Figures 1 and 2 show the mean conditions in the 2015 and 2016 fall growing seasons, respectively. Generally, the 2015 fall growing season was wetter and warmer than the 2016 fall growing season.

The experimental design of each field trial was a randomized block design consisting of four N fertilizer rates replicated four times for a total of 16 plots. Fertilizer rates of 0, 45, 90, and 134 kg N ha<sup>-1</sup> were applied to plots  $3 \times 6$  m in size (except one trial had rates of 0, 56, 112, and 168 kg N ha<sup>-1</sup>). Dry urea granules were weighed into sealable plastic bags and spread 19 Aug. to 4 Sept. 2015 and from 30 Aug. to 15 Sept. 2016 by hand. The central 75% of dates was 31 August to 4 September. When necessary, a temporary fence was erected around the area of ~18  $\times$  30 m so that fertilizer and grazing animals could be kept off the plot area until harvest in winter.

#### **Plant and Soil Analyses**

Harvest of fall-stockpiled forage occurred from 11 Dec. 2015 to 21 Jan. 2016 and from 2 Dec. 2016 to 26 Jan. 2017. The central 75% of dates across years was 12 December to 18 January. A rotary mower with rear vacuum bag (cutting width, 0.5 m) was used to collect forage. During the first year, only forage  $\geq 10$  cm height was collected. During the second year, forage was first collected at  $\geq 10$  cm height, and then a second cutting at 5-cm height was collected to quantify if yield response to N fertilizer input might also occur in this layer (which we felt could be a layer some producers allow their stock to graze). Forage from an area of 2  $\times$  5 m within each plot was collected and weighed in the field (nearest 0.01 kg). A representative subsample (200–500 g) was collected and weighed at field moisture. Subsamples were then oven-dried at 55°C for  $\geq 3$  d to constant weight to determine DM and moisture content of forage at time of harvest.

Dried forage samples were ground in a Wiley mill to <1 mm particle size. Forage was scanned with near-infrared spectroscopy (Model 5000 NIRS with WinISI version 1.5 software; Foss

Table	I. Location, soil	taxonomy, managemer	nt history, elevation,	and precipitation	of field trials in autumn	2015 and 2016.

State/county/location code	i Soil taxonomy†	Tall fescue type/management/history‡	Elevation	Precipitation (Sept.–Nov.)
			m.a.s.l.	mm
Coastal Plain region NC Johnston (104-CCRS)	Goldsboro SL (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults)/Wedowee SL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, mowed sod with occasional haying, swine slurry in 2014, >20-yr-old conservation reserve sod	98	438
NC Pender (033-LBZZ)	Lumbee L (Fine-loamy over sandy or sandy-skeletal, siliceous, subactive, thermic Typic Endoaquults)/Johns fSL (Fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Aquic Hapludults)	EN, rotationally grazed, 4.5 Mg ha <sup>-1</sup> poultry litter in fall, previously in wild tall fescue (smothered with pearl millet in summer), >10-yr-old pasture	8	460
NC Pender (110-LBEF)	Aycock L (Fine-silty, siliceous, subactive, thermic Typic Paleudults)	EN, rotationally grazed, poultry litter in fall, 5-yr-old pasture	15	662
NC Pender (122-LBWF)	Exum L (Fine-silty, siliceous, subactive, thermic Aquic Paleudults)	EN, rotationally grazed, poultry litter in fall, 5-yr-old pasture	15	662
NC Wayne (034-CRFS)	Lumbee SL (Fine-loamy over sandy or sandy-skeletal, siliceous, subactive, thermic Typic Endoaquults)	EN since 2015, rotationally grazed + hayed, 130–150 kg N ha <sup>-1</sup> yr <sup>-1</sup> in three splits, tilled in 2015 for renovation, >10-yr-old pasture	22	613
NC Wayne (035-CRFD)	Weston LS (Coarse-loamy, siliceous, semiactive, thermic Typic Paleaquults)/Leaf L (Fine, mixed, active, thermic Typic Albaquults)	EN since 2015, rotationally grazed + hayed, 130–150 kg N ha <sup>-1</sup> yr <sup>-1</sup> in three splits, tilled in 2015 for renovation, >10-yr-old pasture	22	613
NC Wayne (105-CRFZ	Johns SL (Fine-Ioamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Aquic Hapludults)	EN, hayland, routine inorganic fertilization, I-yr-old, previously in EW pasture	20	497
Piedmont region				
GA Oconee (016-UGAZ)	Cecil overwash (Fine, kaolinitic, thermic Typic Kanhapludults)/Pacolet SCL (Fine, kaolinitic, thermic Typic Kanhapludults)	EVV, occasionally grazed + mowed, limited fertilization, >50-yr-old pasture	237	89
GA Oglethorpe (017-HTZZ)	Cecil SL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, 20% bermudagrass, rotationally grazed, 4.5 Mg ha <sup>-1</sup> broiler litter in fall, 12 yr ago with brush removal of old field, >40-yr-old pasture	217	89
GA Oglethorpe (018-WFZZ)	Appling cSL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, rotationally grazed, 6.7 Mg ha <sup>-1</sup> broiler litter in fall, previously in forest, 4-yr-old pasture	214	89
GA Wilkes (019-WHZZ)	Georgeville CL (Fine, kaolinitic, thermic Typic Hapludults)	EW, 25% bermudagrass, rotationally grazed + hay every 3 yr, 50–70 kg N ha <sup>-1</sup> routinely in fall, >40-yr-old pasture	165	56
NC Durham (031-BCCZ)	Helena SL (Fine, mixed, semiactive, thermic Aquic Hapludults)	EN since 2013, hayed in 2014, rotationally grazed, 70 kg N ha <sup>-1</sup> in spring and 36–36–36 kg NPK ha <sup>-1</sup> in fall, previously in corn grain with no-till, 3-yr-old pasture	140	342
NC Durham (102-BCC1)	Georgeville SiL (Fine, kaolinitic, thermic Typic Kanhapludults)/Lignum SiL (Fine, mixed, semiactive, thermic Aquic Hapludults)	EW, rotationally grazed, >10-yr-old pasture	138	390
NC Durham (103-BCCN)	Helena SL (Fine, mixed, semiactive, thermic Aquic Hapludults)	EN, I-yr-old stand, following grain crops	137	390
NC Granville (032-LDZZ)	Georgeville SiL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, rotationally grazed, occasional clover overseeding, routine inorganic fertilization, 29-yr-old pasture	152	356
NC Granville (113-LDZZ)	Georgeville SiL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, mixed stand, rotationally grazed + mowed, 19–19–19 kg NPK ha <sup>-1</sup> yr <sup>-1</sup> , 28-yr-old pasture	152	446
NC Guilford (027-YDZZ)	Vance SL (Fine, mixed, semiactive, thermic Typic Hapludults)	EW, 60% other forages, rotationally grazed for 4 yr, biosolids at 1.7 Mg ha <sup><math>-1</math></sup> in spring and fall (6% NP), inorganically fertilized hay previously for 10 yr, tobacco 15 yr ago, 14-yr-old pasture	228	265
NC Montgomery (107-SRSZ)	Candor S (Sandy, kaolinitic, thermic Grossarenic Kandiudults)	EW, turf mixture, mowed as turf, routine inorganic fertiliza- tion, >10-yr-old sod	177	610
NC Person (030-RJZZ)	Herndon L (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, rotationally grazed, 40–60 kg N ha <sup>-1</sup> in fall, >25-yr-old pasture	169	358
NC Person (116-RJZZ)	Herndon L (Fine, kaolinitic, thermic Typic Kanhapludults)/Enon fSL (Fine, mixed, active, thermic Ultic Hapludalfs)	EW, rotationally grazed, routine fall fertilization, >10-yr-old pasture	162	446
NC Randolph (026-PRZZ)	Georgeville SiCL (Fine, kaolinitic, thermic Typic Kanhapludults)	EW, rotationally grazed, >10-yr-old pasture	171	184
NC Rockingham (028-BJZZ)	Clover SCL (Fine, mixed, semiactive, mesic Typic Hapludults)	EW, 20% clover, 20% crabgrass, 10% purpletop, rotationally grazed, not typically fertilized, >20-yr-old pasture	183	255

State/county/location	<u>,                                     </u>			Precipitation
code	Soil taxonomy†	Tall fescue type/management/history‡	Elevation	(Sept.–Nov.)
NC Rockingham (029-UPRS)	Rhodiss SL (Fine-Ioamy, mixed, semiactive, mesic Typic Hapludults)	EW, 15% other forages, rotationally grazed + mowed, 40 kg N ha <sup>-1</sup> in spring, >50-yr-old pasture	256	255
NC Rockingham (114-UPRM)	Casville SL (Fine, mixed, semiactive, mesic Typic Hapludults)	EW, mixed stand, mowed as turf since 15 yr with no fertil- ization previously pasture for $>10$ yr	266	173
NC Rockingham (115-UPRG)	Rhodiss SL (Fine-loamy, mixed, semiactive, mesic Typic Hapludults)	EW, rotationally grazed, routinely 44 kg N ha <sup>-1</sup> in spring, >50-yr-old pasture	256	173
NC Rowan (020-PRSN)	Lloyd CL (Fine, kaolinitic, thermic Rhodic Kanhapludults	EN, hayed, routine inorganic fertilization, previously in grain production with no-till, 2-yr-old hayland	219	190
NC Rowan (021-PRSO)	Mecklenburg CL (Fine, mixed, active, thermic Ultic Hapludalfs	EN, hayed, routine inorganic fertilization, previously in grain production with no-till, 5-yr-old hayland	219	190
NC Rowan (117-PRSL)	Dorian fSL (Fine, mixed, semiactive, thermic Aquic Hapludults)	EW, mixed stand, occasionally grazed + hayed + mowed, no fertilization, >50-yr-old waterway	208	645
NC Rowan (118-PRSU)	Lloyd CL (Fine, kaolinitic, thermic Rhodic Kanhapludults)	) EN, cut for hay, routine inorganic fertilization, I-yr-old hay field following grain cropping with no-till	219	645
NC Stanly (025-LNZZ)	Tarrus chSiCL (Fine, kaolinitic, thermic Typic Kanhapludults)/Badin chSiL (Fine, mixed, semiactive, thermic Typic Hapludults)	EW, 30% other forages, rotational grazing, 60 kg N ha <sup>-1</sup> in spring and occasionally in fall, previously in woods (1 yr soybean with no-till in 1996), 57-yr-old pasture	99	251
NC Surry (022-JMRE)	Fairview SCL (Fine, kaolinitic, mesic Typic Kanhapludults)	EW, 50% other forages, rotationally grazed, 55 kg N ha <sup>-1</sup> in fall, 26-yr-old pasture	331	68
NC Surry (023–JMBO)	Fairview SCL (Fine, kaolinitic, mesic Typic Kanhapludults)	EN since 2014, 50% orchardgrass + matua + festolium + bluegrass + switchgrass, rotationally grazed, 55 kg N ha <sup>-1</sup> in Aug. 2015 + 60 kg NPK ha <sup>-1</sup> in summer 2016, renovated pasture with 2 yr annual forages for grazing, 10-yr-old pasture	323	68
NC Surry (024-JMBH)	Fairview SCL (Fine, kaolinitic, mesic Typic Kanhapludults)	EW, 30% other forages, rotationally grazed, 55 kg N ha <sup>-1</sup> in fall, 67-yr-old pasture	321	68
NC Surry (108-JMLF)	Arkaqua L (Fine-Ioamy, mixed, active, mesic Fluvaquentic Dystrudepts)/Fairview SCL (Fine, kaolinitic, mesic Typic Kanhapludults)	EW, rotationally grazed, 56–84 kg N ha <sup>-1</sup> yr <sup>-1</sup> in fall, I4-yr- old pasture	305	636
NC Surry (109-JMRW)	Fairview SCL (Fine, kaolinitic, mesic Typic Kanhapludults)	EW, rotationally grazed, 56–84 kg N ha <sup>-1</sup> yr <sup>-1</sup> in fall, 14-yr- old pasture	330	636
NC Wake (121-LWRZ)	Cecil SL (Fine, kaolinitic, thermic Typic Kanhapludults)/ Chewacla SCL (Fine-Ioamy, mixed, active, thermic Fluvaquentic Dystrudepts)	EW, rotationally grazed, routine inorganic fertilization, >10-yr-old pasture	125	458
VA Culpeper (008-WFZZ)	Cecil fSL (Fine, kaolinitic, thermic Typic Kanhapludults)/ Louisburg SL (Coarse-Ioamy, mixed, semiactive, thermic Ruptic-Ultic Dystrudepts)	EW, 25% other forages including clover, rotationally grazed, 4.5 Mg ha <sup>-1</sup> broiler litter every 3 yr, >50-yr-old pasture	169	105
VA Fauquier (007-RFZZ)	Myersville SiL (Fine-loamy, mixed, active, mesic Ultic Hapludalfs)	EW, rotationally grazed, occasional liming and PK by soil test. >10-yr-old pasture	, 230	169
VA Goochland (011-NRZZ)	Monacan complex (Fine-loamy, mixed, active, thermic Fluvaquentic Eutrudepts)/Madison CL (Fine, kaolinitic, thermic Typic Kanhapludults	EW, 35% other forages, rotationally grazed, 55 kg N ha <sup>-1</sup> yr <sup>-1</sup> in fall, >40-yr-old pasture	50	256
VA Halifax (012-MMZZ)	Clifford SL (Fine, kaolinitic, mesic Typic Kanhapludults)	EW, 10% other forages, rotationally grazed + occasional hay, 22–33–67 kg NPK ha <sup>-1</sup> in fall + 67 kg N ha <sup>-1</sup> in spring (67 kg N ha <sup>-1</sup> in fall if stockpiled), 12-yr-old pasture	192	228
VA Loudoun (101-LCZZ)	Middleburg SiL (Fine-Ioamy, mixed, mesic Ultic Hapludalfs)/Brumbaugh cobbly SiL (Fine-Ioamy, mixed, semiactive, mesic Oxyaquic Hapludults)	EW, mixed stand, rotationally grazed, routine inorganic fertil- ization, >30-yr-old pasture	187	246
Ridge and Valley region	on			
NC Ashe (013-UMRS)	Toxaway L (Fine-loamy, mixed, superactive, nonacid, mesic Cumulic Humaquepts)/Watauga L (Fine-loamy, paramicaceous, mesic Typic Hapludults)	EW 75%, mixed stand with clover, orchardgrass, and timothy, rotationally grazed + mowed, 56–56–56 kg NPK ha <sup>-1</sup> yr <sup>-1</sup> , >30-yr-old pasture	868	250
NC Ashe (111-UMRG)	Toxaway L (Fine-Ioamy, mixed, superactive, nonacid, mesic Cumulic Humaquepts)	EW, mixed stand, occasionally grazed, >40-yr-old pasture	867	716
NC Ashe (112-UMRW)	Watauga L (Fine-Ioamy, paramicaceous, mesic Typic Hapludults)	EW, mixed stand, rotationally grazed, >40-yr-old pasture	871	716
NC Clay (015-HBZZ)	French fSL (Fine-loamy over sandy or sandy-skeletal, mixed, active, mesic Fluvaquentic Dystrudepts)	EW, 25% mixed forages, rotationally grazed, 9 Mg ha <sup>–1</sup> chicken litter every 5 yr, >40-yr-old-pasture	512	70

Continued

State/county/location			14	Precipitation
code	Soil taxonomy†	Tall fescue type/management/history‡	Elevation	(Sept.–Nov.)
NC Clay (106-HBZZ)	Brasstown-Junaluska complex (Fine-Ioamy, mixed, subactive, mesic Typic Hapludults)	EW, rotationally grazed, poultry litter every 5 yr, >40-yr-old pasture	530	322
NC Haywood (014-MRSZ)	Braddock CL (Fine, mixed, subactive, mesic Typic Hapludults)/Saunook L (Fine-Ioamy, mixed, superactive, mesic Humic Hapludults)	EW, rotationally grazed, routine inorganic fertilization in spring, 28-yr-old pasture	834	177
NC Haywood (119-MRSL)	Cullowhee-Nikwasi complex (Coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Fluvaquentic Humudepts; nonacid, mesic Cumulic Humaquepts	EW, mixed stand, rotationally grazed, infrequent fertilization, >30-yr-old pasture	812	606
NC Haywood (120-MRSU)	Braddock CL (Fine, mixed, subactive, mesic Typic Hapludults)	EW, rotationally grazed, infrequent fertilization, >30-yr-old pasture	822	606
VA Augusta (009-SVAI)	Frederick-Christian SiL (Fine, mixed, semiactive, mesic Typic Paleudults;Typic Hapludults)	EW,~10% other forages, rotationally grazed, 48–123–0 kg NPK ha <sup>-1</sup> in spring 2016, >50-yr-old pasture	580	258
VA Augusta (010-SVAU)	Frederick-Christian SiL (Fine, mixed, semiactive, mesic Typic Paleudults;Typic Hapludults)	EW, ~10% other forages, rotationally grazed, typically no fertilization other than occasional P and K, >50-yr-old pasture	579	258
VA Carroll (004-SMR1)	Myersville L (Fine-Ioamy, mixed, active, mesic Ultic Hapludalfs)/Hayesville L (Fine, kaolinitic, mesic Typic Kanhapludults)	EN since 2014, 33% red/white clover, rotationally grazed + cut for hay, occasional PK fertilization in spring, >10-yr-old pasture	767	135
VA Carroll (005-SMHP)	Manor L (Coarse-loamy, micaceous, mesic Typic Dystrudepts)/Hayesville L (Fine, kaolinitic, mesic Typic Kanhapludults)	EN since 2012, 33% red/white clover, rotationally grazed + cut for hay, occasional PK fertilization in spring, >15-yr-old pasture	807	135
VA Carroll (006-SMWL)	Myersville L (Fine-Ioamy, mixed, active, mesic Ultic Hapludalfs)	EN since 2011, 33% red/white clover, rotationally grazed + cut for hay, occasional PK fertilization in spring, >30-yr-old pasture	759	135
VA Pulaski (003-SBZZ)	Lowell SiL (Fine, mixed, active, mesic Typic Hapludalfs)	EW, 50% other forages, rotationally grazed + occasional hay, no fertilization, >10-yr-old pasture	638	102
WV Monongalia (001-KFIM)	Ernest SiL (Fine-loamy, mixed, superactive, mesic Aquic Fragiudults)	EW, 70% clover + indiangrass, hayed twice per year, routine NPK in spring, >10-yr-old hay field	456	247
WV Monongalia (002-KFUN)	Gilpin SiL (Fine-loamy, mixed, active, mesic Typic Hapludults)/Ernest SiL (Fine-loamy, mixed, superactive, mesic Aquic Fragiudults)	EW, 30% indiangrass, hayed once per year, no fertilization, >10-yr-old hay field	471	247

† chSiCL, channery silty clay loam; chSiL, channery silt loam; CL, clay loam; cSL, coarse sandy loam; fSL, fine sandy loam; L, loam; LS, loamy sand; S, sand; SCL, sandy clay loam; SiCL, silty clay loam; SiL, silt loam; SL, sandy loam.

‡ EN, endophyte-infected tall fescue with novel strain; EW, endophyte-infected tall fescue with wild type.

North America, Inc., Eden Prairie, MN) to predict C and N concentrations. Calibration was by evaluating spectra for outliers ('H' >3.0) prior to sample selection for chemical determinations. An 'H' statistic of 0.6 was used to select samples with different spectra. The total number of samples selected with different spectra was 90 out of a possible 320 (20 trials × 16 plots/trial) in 2015 and 189 out of a possible 1120 (35 trials × 16 plots/trial × 2 layers/plot) in 2016. Selected samples were analyzed for C and N concentration with a CN combustion analyzer (TruMac; Leco Corp., St. Joseph, MI). Equations were developed for calibrating spectra to C and N concentration of dried forage using modified partial least squares regression with four cross validations. Standard error of calibration was 3.0 mg C g<sup>-1</sup> forage DM (CV, 0.7%) in both 2015 and 2016 and 0.34 mg N  $g^{-1}$ forage DM (CV, 1.5%) in 2015 and 0.44 mg N  $g^{-1}$  forage DM (CV, 2.1%) in 2016. The standard error of cross validation was  $3.7 \text{ mg C g}^{-1}$  forage DM (CV, 0.8%) and 0.47 mg N g $^{-1}$  forage DM (CV, 2.1%) in 2015 and 4.5 mg C g<sup>-1</sup> forage DM (CV, 1.0%) and 0.51 mg N  $g^{-1}$  forage DM (CV, 2.5%) in 2016. The library of 90 samples in 2015 was added to the library in 2016 to predict concentrations in 2016. The range of C concentration was 415 to 493 mg C  $g^{-1}$  forage DM, and the range of N concentration was

8.0 to 35.2 mg N  $g^{-1}$  forage DM. Total N uptake of forage was calculated from the product of DM and N concentration.

Soil was sampled in each field trial the same day or within a couple of weeks prior to fertilization. Sampling occurred from 5 Aug. to 3 Sept. 2015 and from 10 Aug. to 15 Sept. 2016. The central 75% of dates across years was 11 August to 1 September. Within each of the four blocks of a field trial, eight soil cores (4-cm diameter) at depth of 0 to 10 cm were composited in a paper bag and subjected to enhanced drying within 12 h of collection. Due to the number of fields to be sampled, some soils were initially air-dried with a fan blowing across them, and all soils were eventually oven-dried in a forced-air oven at 55°C for  $\geq 3$  d. Soil samples were homogenized by gently crushing with a pestle over a sieve with 4.75-mm openings. Stones and pieces of organic residue not passing the screen were discarded.

Soil was analyzed on the <4.75-mm samples for most properties. Only for total organic C (TOC) and total soil N (TSN) and initial inorganic N was a subsample ground to a fine powder in a ball mill. Total organic C and TSN were determined with dry combustion using a Leco TruMac CN analyzer. Routine soil nutrient analyses were conducted by Soil Testing Services of the North Carolina Department of Agriculture and Consumer



Fig. 1. Mean weekly weather conditions across 15 locations during the 2015 fall growing season. The value next to precipitation bars is the actual precipitation, which exceeded the 50-mm limit in the graph.

Services in Raleigh NC. Soil pH was from 1:2 (v/v) of soil/water with glass electrode. Concentrations of Ca, Cu, K, Mg, Mn, Na, P, S, and Zn were determined with Mehlich-III extraction followed by determination with inductively coupled spectroscopy.

Soil organic C and N fractions were determined according to Franzluebbers and Stuedemann (2008). Briefly, soil microbial biomass C was determined with chloroform fumigation-incubation without subtraction of a control and using an efficiency factor of 0.41 (Franzluebbers et al., 1999; Voroney and Paul, 1984). The flush of  $CO_2$  following rewetting of dried soil (3 d) and cumulative C and N mineralization during 24 d of incubation were determined with aerobic incubation of soil at 50% water-filled pore space and 25°C. Duplicate 50-g soil samples in 60-mL glass jars were wetted and placed in a 1-L canning jar along with a vial containing 10 mL of 1 mol L<sup>-1</sup> NaOH to trap CO<sub>2</sub> and a vial of water to maintain humidity. Alkali traps were replaced at 3 and 10 d of incubation and CO<sub>2</sub>-C determined by titration with 1 mol L<sup>-1</sup> HCl with vigorous stirring in the presence of  $BaCl_2$  (which precipitated to form  $BaCO_3$ ) to a phenolphthalein endpoint. At 10 d, one of the subsamples was removed from the incubation jar and fumigated with CHCl<sub>2</sub> under vacuum for 1 d, vapors removed, placed into a separate canning jar along with vials of alkali and water, and incubated at 25°C for 10 d. Potential C mineralization was calculated from the cumulative evolution of CO2 during 24 d of incubation. Basal soil respiration was assumed from the linear rate of C mineralization during the 10- to 24-d period. Mineralizable N was determined from the difference in inorganic N concentration between 0 and 24 d of incubation. Inorganic N (NH<sub>4</sub>-N  $+ NO_2 - N + NO_3 - N$ ) was determined from the filtered extract



Fig. 2. Mean weekly weather conditions across 24 locations during the 2016 fall growing season. The value next to precipitation bars is the actual precipitation, which exceeded the 50-mm limit in the graph.

of a 10-g subsample of dried (55°C for 3 d) and sieved (≤2 mm) soil that was shaken with 20 mL of 2 mol L<sup>-1</sup> KCl for 30 min using salicylate-nitroprusside and hydrazine autoanalyzer techniques (Bundy and Meisinger, 1994). Plant-available N was calculated on an area basis (kg N ha<sup>-1</sup>) as the summation of residual inorganic N (NO<sub>3</sub> + NH<sub>4</sub>) and mineralizable N during 24 d of incubation, which were multiplied by bulk density.

Particulate organic matter and soil texture were determined from the dried sample (55°C for 3 d) previously used to estimate soil microbial biomass C (Franzluebbers and Stuedemann, 2008). A 50-g soil sample was shaken with 100 mL of 0.1 mol  $L^{-1}$  Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> for 16 h and then diluted in a 1-L volumetric cylinder with deionized water. The dilute soil solution was mixed with a plunger 10 times and allowed to settle for exactly 5 h, at which time a hydrometer was inserted to determine the density of the solution as a proxy for clay concentration (Gee and Bauder, 1986). The soil–solution mixture was passed over a sieve with 0.053-mm openings to collect the sand fraction (>0.053 mm), which was dried (55°C for 24 h past visual dryness), weighed, ball milled, and analyzed for C and N with dry combustion as described for TOC and TSN. Silt concentration was estimated from the difference between unity and fractions of clay and sand.

#### **Statistical Analyses**

Forage response variables were regressed on the four continuous N rates using all four replications in each of the 55 field trials. Response variables were DM and total N uptake for the >10-cm layer in 2015 and DM and total N uptake for the >10cm, 5- to 10-cm, and >5-cm layers in 2016. All response variables were first tested for fit to a nonlinear function of the form:

$$DM = DM_0 + a \cdot (1 - e^{-b \cdot N})$$

where, DM is dry matter (Mg ha<sup>-1</sup>), DM<sub>0</sub> is baseline DM yield without N (Mg ha<sup>-1</sup>), *a* is the additional yield potential with limitless N input (Mg ha<sup>-1</sup>), *b* is the nonlinear rate constant, and N is fertilizer N rate (kg N ha<sup>-1</sup>). When the nonlinear equation produced either a negative DM response or a nearly vertical rise at the first instance of N input followed by no change thereafter, then a linear regression was fitted to the data. If the linear regression had negative slope, then mean DM across N rates was calculated to assume no response to N input. These were the only three choices used to calculate the following parameters of interest for further statistical evaluation of each site: (i) maximum DM yield based on regression at the highest N rate tested (Mg ha<sup>-1</sup>), (ii) relative DM yield without N fertilizer derived from the best-fit regression equation at 0 kg N ha<sup>-1</sup> divided by maximum DM yield (Mg Mg<sup>-1</sup>), (iii) DM yield response to initial dose of N (empirically derived from the instantaneous DM yield produced at the first instance of N; i.e., in nonlinear form, this equated to regression parameters a \* b, and in linear form this was the slope parameter) based on best-fit regression (kg DM kg<sup>-1</sup> N), (iv) economically optimum N fertilizer requirement (EONR) at low cost-to-value ratio (kg N ha<sup>-1</sup>) (EONR was the point at which the slope between yield and N rate was equal to the cost-to-value threshold: N rates > EONR produced forage DM yield that had less value than the cost of N fertilizer), (v) EONR at medium cost-to-value ratio (kg N ha<sup>-1</sup>), and (vi) EONR at high cost-to-value ratio (kg N ha<sup>-1</sup>).

Threshold cost-to-value ratios were calculated from the cost of N fertilizer ( $\$ kg^{-1}$ ) and the value of forage ( $\$ kg^{-1}$ ). The low-threshold cost-to-value ratio was calculated at the equivalent of  $\$1.00 kg^{-1}$  N and  $\$0.20 kg^{-1}$  forage (= 5 kg forage DM kg<sup>-1</sup> N). The high-threshold cost-to-value ratio was calculated at the equivalent of  $\$2.00 kg^{-1}$  N and  $\$0.10 kg^{-1}$  forage (= 20 kg forage DM kg<sup>-1</sup> N). The medium threshold cost-to-value ratio was calculated similarly for a target of 10 kg forage DM kg<sup>-1</sup> N. These three targets of EONR (kg N ha<sup>-1</sup>) were also calculated as N factor for economically optimum production (kg N Mg<sup>-1</sup> forage DM). Similar regressions and calculations were performed with total N uptake and setting arbitrary levels of 0.10, 0.25, and 0.50 kg N uptake kg<sup>-1</sup> N applied as low, medium, and high thresholds of efficiency.

After regression calculations, forage responses were single observations for each harvest layer in each field trial. Soil variables were also averaged across replications to associate with plant variables in each field trial. Standard deviations of soil variables among blocks within a field trial were calculated for each of the 57 trials. Linear and nonlinear regressions among plant and soil variables were performed with means from each field trial using SAS v. 9.4 and SigmaPlot v. 13. To show more clearly the trends in forage DM and N uptake response to N fertilizer application amid the large field variations that occurred, the means of five consecutive field trials in ranked order of soil-test biological activity were calculated and used in linear regressions (n = 5 field trials in each group). Regressions for the  $\geq$ 10-cm forage layer were combined across years (*n* = 55 overall or n = 45 when drought-affected field trials deleted), and data for the 5- to 10-cm layer and total harvest (≥5-cm height) were analyzed for 2016 only (n = 35 overall or n = 25 when droughtaffected field trials deleted). Effects were considered significant



Fig. 3. Soil texture of 0- to 10-cm depth of 57 field trials on tall fescue pasture.

at  $p \le 0.05$ . Significance of correlations among variables was set at a stricter threshold of  $p \le 0.01$  to avoid spurious associations.

## **RESULTS AND DISCUSSION**

#### Soil Characteristics

Soil texture of the 57 fields was distributed widely among eight classes (Fig. 3). Loam was the most populated class (32% of fields), followed by sandy loam (25% of fields), clay loam (14% of fields), and silt loam (12% of fields). A Piedmont field in Rowan County, NC, had the highest clay concentration (415 g kg<sup>-1</sup>), and a Piedmont field in Montgomery County, NC, had the lowest clay concentration (67 g kg<sup>-1</sup>) and silt concentration (55 g kg<sup>-1</sup>); this was also the field with the highest sand concentration (878 g kg<sup>-1</sup>). Sand concentration was lowest at a Ridge and Valley field in Monongalia County, WV (128 g kg<sup>-1</sup>). Silt concentration was greatest at a Ridge and Valley field in Augusta County, VA (663 g kg<sup>-1</sup>).

Total organic C and TSN at depth of 0 to 10 cm averaged 26.5 and 2.4 g kg<sup>-1</sup>, respectively, among the 57 fields (Table 2). Total organic C was as low as 13.4 g kg<sup>-1</sup> in a loamy fine sand in Montgomery County, NC, and as high as 56.0 g kg<sup>-1</sup> in a clay loam in Ashe County, NC. Total soil N was as low as 0.87 g kg<sup>-1</sup> in a sandy loam in Oglethorpe County, GA, and as high as 4.60 g kg<sup>-1</sup> in a loam in Ashe County, NC. Total organic C tended to be greater with increasing elevation ( $r^2 = 0.31$ ; p < 0.001), which was partially attributable to generally cooler temperature and somewhat greater clay concentration in soil  $(r^2 = 0.16; p = 0.002)$ . Total organic C averaged 19.6 g kg<sup>-1</sup> in the Coastal Plain, 24.4 g kg<sup>-1</sup> in the Piedmont, and 34.1 g kg<sup>-1</sup> in the Ridge and Valley regions. Total organic C in tall fescue pastures of this study was generally greater than reported for no-tillage and organic crop production in the Piedmont and mountains of North Carolina (13–19 g kg<sup>-1</sup>) (Muruganandam et al., 2009; Wang et al., 2011).

Total soil N was very closely associated with TOC:

 $TOC = 2.5 + 10.7 * TSN, r^2 = 0.91, p < 0.001$ 

Table 2. Mean ( $\pm$  standard deviation) of soil chemical properties at depth of 0–10 cm at each individual field and across regions. Standard deviation is among replications for each field and among fields for each region.

	Soil chemical properties†												
							Extr	actable					
State/county/location code	TOC	TSN	pH‡	CEC	BS	Р	K Ca		Mg				
	——— g k	'g <sup>-1</sup>		cmol <sub>c</sub> kg <sup>-1</sup>	%		mg	dm <sup>-3</sup>					
Coastal Plain region													
NC Johnston (104-CCRS)	19.7 (1.4)	1.62 (0.13)	5.6 (0.1)	7.3 (0.4)	78 (4)	43 (4)	130 (23)	778 (130)	175 (15)				
NC Pender (033-LBZZ)	37.8 (2.9)	2.12 (0.12)	6.1 (0.1)	11.2 (1.0)	84 (2)	88 (22)	98 (30)	1538 (175)	178 (17)				
NC Pender (110-LBEF)	17.5 (1.0)	1.11 (0.06)	6.1 (0.1)	7.4 (0.4)	85 (2)	103 (4)	87 (11)	1039 (77)	107 (4)				
NC Pender (122-LBWF)	18.3 (0.4)	1.22 (0.04)	6.2 (0.2)	7.9 (0.9)	87 (3)	133 (26)	129 (25)	1080 (196)	133 (15)				
NC Wayne (034-CRFS)	13.8 (1.1)	1.14 (0.09)	5.8 (0.1)	5.9 (0.5)	77 (2)	205 (33)	110 (14)	655 (74)	117 (16)				
NC Wayne (035-CRFD)	15.8 (1.7)	1.31 (0.17)	6.1 (0.4)	7.3 (1.8)	85 (10)	152 (5)	84 (13)	933 (13)	179 (69)				
NC Wayne (105-CRFZ	14.6 (1.2)	1.27 (0.09)	6.3 (0.3)	8.5 (1.2)	89 (5)	35 (3)	51 (1)	1061 (186)	263 (62)				
Coastal Plain mean	19.6 (8.3)	1.40 (0.36)	6.0 (0.2)	7.9 (1.7)	83 (4)	108 (60)	98 (28)	1012 (281)	164 (53)				
Piedmont region													
GA Oconee (016-UGAZ)	34.7 (9.4)	3.00 (0.91)	6.1 (0.1)	10.7 (1.9)	86 (2)	171 (78)	260 (67)	1335 (282)	223 (31)				
GA Oglethorpe (017-HTZZ)	24.5 (4.3)	2.00 (0.33)	6.3 (0.1)	9.4 (1.7)	88 (3)	153 (30)	204 (56)	1237 (259)	201 (42)				
GA Oglethorpe (018-WFZZ)	16.2 (3.5)	0.87 (0.13)	6.1 (0.1)	5.7 (1.0)	81 (4)	85 (6)	139 (38)	671 (145)	111 (25)				
GA Wilkes (019-WHZZ)	26.1 (1.1)	2.03 (0.14)	5.8 (0.1)	7.2 (1.1)	80 (2)	46 (4)	124 (29)	892 (125)	115 (37)				
NC Durham (031-BCCZ)	16.7 (1.5)	1.42 (0.11)	6.1 (0.2)	6.5 (0.6)	82 (4)	70 (2)	33 (I)	724 (98)	197 (31)				
NC Durham (102-BCC1)	25.6 (1.4)	2.27 (0.13)	6.1 (0.3)	13.3 (0.9)	90 (4)	72 (20)	98 (17)	1561 (160)	478 (55)				
NC Durham (103-BCCN)	14.8 (0.2)	1.31 (0.02)	6.5 (0.1)	7.9 (0.4)	9I (I)	81 (5)	50 (15)	940 (35)	280 (12)				
NC Granville (032-LDZZ)	19.2 (3.8)	1.67 (0.32)	5.8 (0.1)	7.2 (1.7)	81 (5)	49 (11)	189 (36)	733 (229)	208 (68)				
NC Granville (113-LDZZ)	20.6 (1.6)	1.71 (0.15)	6.1 (0.4)	11.0 (1.6)	88 (4)	36 (14)	92 (18)	1300 (94)	349 (78)				
NC Guilford (027-YDZZ)	16.0 (1.3)	1.38 (0.12)	6.6 (0.1)	9.1 (2.2)	93 (3)	144 (41)	70 (14)	1269 (352)	244 (56)				
NC Montgomery (107-SRSZ)	13.4 (1.2)	1.02 (0.10)	6.3 (0.1)	5.9 (0.4)	83 (I)	126 (18)	49 (5)	630 (50)	200 (7)				
NC Person (030-RJZZ)	26.4 (3.5)	2.18 (0.34)	5.5 (0.2)	10.5 (3.1)	79 (8)	57 (12)	85 (29)	1092 (381)	338 (145)				
NC Person (116-RJZZ)	28.1 (1.7)	2.48 (0.18)	5.6 (0.1)	9.5 (0.3)	81 (2)	97 (5)	108 (25)	960 (58)	307 (21)				
NC Randolph (026-PRZZ)	19.6 (2.4)	1.65 (0.19)	6.1 (0.1)	8.4 (1.6)	83 (4)	58 (7)	215 (45)	967 (247)	199 (48)				
NC Rockingham (028-BJZZ)	31.8 (2.9)	2.98 (0.28)	6.3 (0.1)	19.8 (2.6)	92 (2)	15 (2)	372 (106)	2871 (465)	350 (53)				
NC Rockingham (029-UPRS)	22.6 (1.7)	2.03 (0.16)	7.1 (0.3)	14.3 (2.6)	98 (3)	102 (13)	149 (42)	2616 (593)	83 (24)				
NC Rockingham (114-UPRM)	26.2 (2.8)	2.40 (0.24)	5.8 (0.1)	8.6 (0.7)	82 (3)	80 (18)	167 (24)	1042 (121)	171 (15)				
NC Rockingham (115-UPRG)	23.9 (1.7)	2.21 (0.21)	7.1 (0.6)	14.3 (5.0)	97 (6)	117 (19)	166 (30)	2552 (1106)	99 (19)				
NC Rowan (020-PRSN)	17.5 (0.3)	1.54 (0.01)	5.7 (0.1)	7.3 (1.2)	74 (6)	131 (7)	74 (15)	714 (176)	210 (45)				
NC Rowan (021-PRSO)	21.8 (1.9)	2.08 (0.12)	7.0 (0.1)	23.4 (3.7)	98 (I)	369 (34)	356 (77)	3697 (551)	420 (107)				
NC Rowan (117-PRSL)	36.5 (3.0)	3.54 (0.37)	5.9 (0.1)	11.9 (1.0)	87 (2)	142 (39)	499 (124)	1355 (106)	282 (33)				
NC Rowan (118-PRSU)	16.6 (1.1)	1.48 (0.09)	5.6 (0.2)	7.5 (0.6)	76 (I)	174 (4)	96 (22)	751 (93)	203 (27)				
NC Stanly (025-LNZZ)	34.0 (10.3)	2.92 (0.91)	6.7 (0.1)	19.2 (1.6)	95 (I)	398 (63)	266 (29)	2709 (259)	492 (63)				
NC Surry (022-JMRE)	26.1 (0.6)	1.87 (0.07)	6.9 (0.1)	12.2 (2.0)	95 (2)	34 (6)	162 (47)	1516 (228)	449 (102)				
NC Surry (023-JMBO)	25.8 (4.4)	2.07 (0.33)	6.9 (0.1)	13.9 (1.4)	96 (2)	150 (44)	214 (87)	1884 (207)	413 (39)				
NC Surry (024-JMBH)	33.3 (2.8)	2.92 (0.29)	6.5 (0.1)	13.4 (0.9)	9I (I)	90 (28)	223 (53)	1558 (69)	469 (59)				
NC Surry (108-JMLF)	26.9 (4.6)	2.00 (0.19)	6.5 (0.1)	10.7 (1.5)	91 (3)	72 (7)	202 (44)	1285 (183)	344 (64)				
NC Surry (109-JMRW)	29.5 (1.9)	2.13 (0.11)	7.0 (0.1)	14.5 (1.2)	97 (I)	76 (24)	191 (29)	1852 (213)	533 (35)				
NC Wake (121-LWRZ)	34.7 (1.2)	2.72 (0.13)	5.9 (0.1)	9.7 (0.2)	83 (3)	58 (21)	76 (16)	1056 (65)	314 (17)				
VA Culpeper (008-WFZZ)	25.2 (2.1)	2.22 (0.18)	5.9 (0.1)	11.2 (1.3)	85 (I)	21 (6)	78 (21)	1493 (196)	228 (33)				
VA Fauquier (007-RFZZ)	31.5 (2.9)	2.99 (0.32)	6.4 (0.1)	20.0 (2.7)	92 (I)	40 (7)	221 (81)	3154 (410)	256 (75)				
VA Goochland (011-NRZZ)	24.4 (0.8)	2.35 (0.09)	5.9 (0.2)	10.5 (1.2)	86 (3)	42 (23)	56 (7)	1519 (266)	161 (20)				
VA Halifax (012-MMZZ)	16.8 (2.0)	1.32 (0.14)	5.5 (0.1)	5.4 (0.4)	71 (5)	91 (11)	75 (6)	534 (78)	122 (19)				
VA Loudoun (101-LCZZ)	21.6 (1.8)	1.90 (0.14)	6.6 (0.1)	11.7 (0.6)	92 (2)	60 (4)	76 (12)	1488 (110)	379 (19)				
Piedmont mean	24.4 (6.4)	2.08 (0.62)	6.2 (0.5)	11.2 (4.4)	87 (7)	103 (83)	160 (104)	1469 (785)	277 (124)				
Ridge and Valley region													
NC Ashe (013-UMRS)	53.3 (11.5)	4.60 (1.12)	5.2 (0.1)	12.4 (2.0)	70 (3)	91 (45)	230 (38)	1186 (294)	252 (44)				
NC Ashe (111-UMRG)	56.0 (3.0)	4.55 (0.20)	5.5 (0.1)	10.5 (0.8)	74 (2)	126 (24)	285 (33)	1029 (102)	232 (26)				
NC Ashe (112-UMRW)	24.2 (1.5)	2.10 (0.17)	5.5 (0.1)	6.5 (0.2)	73 (3)	24 (4)	91 (11)	612 (31)	175 (14)				
NC Clay (015-HBZZ)	31.9 (2.2)	2.91 (0.21)	6.6 (0)	18.7 (0.8)	94 (I)	385 (61)	427 (52)	2880 (163)	265 (31)				
NC Clay (106-HBZZ)	42.1 (3.3)	3.62 (0.25)	6.4 (0.3)	15.2 (0.5)	95 (2)	166 (14)	403 (38)	2252 (167)	251 (23)				
NC Haywood (014-MRSZ)	30.4 (2.6)	2.73 (0.20)	6.4 (0.1)	12.4 (0.8)	91 (1)	91 (10)	139 (32)	1480 (95)	432 (42) Continued				

					F - F				
							Ext	ractable	
State/county/location code	TOC	TSN	PH‡	CEC	BS	P	К	Ca	Mg
NC Haywood (119-MRSL)	33.3 (1.7)	3.11 (0.13)	5.8 (0.1)	12.3 (0.6)	86 (2)	41 (3)	79 (13)	1471 (109)	349 (27)
NC Haywood (120-MRSU)	35.9 (0.9)	3.43 (0.09)	5.9 (0.2)	11.3 (0.9)	85 (2)	86 (10)	166 (85)	1326 (81)	318 (41)
VA Augusta (009-SVAI)	21.2 (2.4)	2.00 (0.17)	6.2 (0.1)	8.8 (0.4)	88 (I)	54 (11)	137 (34)	1097 (37)	232 (30)
VA Augusta (010-SVAU)	22.1 (1.3)	I.88 (0.07)	6.0 (0.1)	6.4 (0.5)	80 (4)	59 (5)	155 (26)	702 (84)	145 (25)
VA Carroll (004-SMR1)	28.3 (2.0)	2.31 (0.20)	6.1 (0.1)	10.0 (1.5)	86 (4)	51 (14)	103 (31)	(199)	343 (70)
VA Carroll (005-SMHP)	30.5 (1.5)	2.71 (0.08)	6.4 (0.2)	13.1 (1.0)	89 (I)	55 (8)	209 (50)	1463 128)	449 (37)
VA Carroll (006-SMVVL)	33.8 (3.0)	2.89 (0.27)	6.0 (0.2)	10.7 (0.3)	84 (I)	63 (12)	223 (40)	1109 (20)	352 (17)
VA Pulaski (003-SBZZ)	24.6 (2.6)	2.20 (0.19)	5.6 (0.2)	8.0 (1.2)	77 (6)	35 (8)	172 (9)	814 (196)	207 (58)
WV Monongalia (001-KFIM)	35.2 (1.1)	2.88 (0.11)	6.0 (0)	12.8 (1.2)	87 (2)	76 (22)	69 (8)	2073 (212)	70 (19)
WV Monongalia (002-KFUN)	43.6 (3.5)	3.29 (0.20)	6.1 (0.2)	14.3 (1.0)	87 (3)	38 (6)	72 (7)	2310 (226)	88 (6)
Ridge and Valley mean	34.1 (10.2)	2.95 (0.82)	6.0 (0.4)	11.5 (3.2)	84 (7)	90 (87)	185 (109)	1432 (638)	260 (110)
				C TCNI	1 1 1 1 1				

Soil chemical properties<sup>†</sup>

† BS, base saturation; CEC, cation exchange capacity; TOC, total organic C; TSN, total soil N.

Table 3. Correlations among soil organic C fractions at depth of 0 to 10 cm across 57 field trials on tall fescue pasture.

Variable†	тос	POC	SMBC	CMIN	BSR	FCO <sub>2</sub>
тос	-	0.54	0.84	0.70	0.61	0.73
POC	***	-	0.58	0.48	0.52	0.43
SMBC	***	***	-	0.84	0.80	0.83
CMIN	***	***	***	-	0.94	0.96
BSR	***	***	***	***	-	0.82
FCO2	***	***	***	***	***	-

\*\*\* Significant at the 0.001 probability level.

<sup>+</sup> BSR, basal soil respiration (mg CO<sub>2</sub>–C kg<sup>-1</sup> soil d<sup>-1</sup>); CMIN, cumulative C mineralization (mg CO<sub>2</sub>–C kg<sup>-1</sup> soil 24 d<sup>-1</sup>), FCO<sub>2</sub>, flush of CO<sub>2</sub> (mg CO<sub>2</sub>–C kg<sup>-1</sup> soil 3 d<sup>-1</sup>); POC, particulate organic C (g kg<sup>-1</sup> soil); SMBC, soil microbial biomass C (mg kg<sup>-1</sup> soil); TOC total organic C (g kg<sup>-1</sup> soil).

This strong relationship was expected because organic matter from plant materials with higher C/N ratio decompose and converge to this stabilized ratio near 10, as has been well documented in many ecosystems around the world (Brady and Weil, 1999). These data from a diversity of fields with tall fescue–dominated pastures provide confirmation for this stable relationship.

With increasing TOC, there were increases in cation exchange capacity (CEC) and extractable K ( $r^2 = 0.23$ ; p < 0.001 in both cases). Curiously, TOC and TSN were not significantly associated with soil pH, base saturation, and extractable P, Ca, and Mg. With increasing clay concentration, there were increases in TOC ( $r^2 = 0.12$ ; p = 0.008), total soil N ( $r^2 = 0.19$ ; p < 0.001), CEC ( $r^2 = 0.17$ ; p = 0.001), extractable K ( $r^2 = 0.14$ ; p = 0.005), and extractable Mg ( $r^2 = 0.11$ , p = 0.01).

Concentration of TOC was also negatively associated with soil bulk density (Fig. 4). This confirms a similar relationship from soils under mixed tall fescue/bermudagrass (*Cynodon dactylon* L.) pasture in Georgia (Franzluebbers and Stuedemann, 2010). Greater TOC reduces bulk density because of the lighter nature of organic matter compared with mineral matter. Soil organic C also feeds a variety of soil organisms, some of which burrow and channel and others that decompose organic matter and glue soil particles into stable aggregates. Therefore, even with trampling by grazing livestock, soil can be spared from compaction with increases in soil organic C, which acts to buffer against compressive forces (Franzluebbers and Stuedemann, 2010; Franzluebbers et al., 2012).



Fig. 4. Association of soil bulk density (BD) with soil organic C (SOC) at a depth of 0 to 10 cm across 57 field trials on tall fescue pasture.

Soil organic C fractions from a diversity of soil types were strongly associated with each other (Table 3), as has been shown previously for Typic Kanhapludults in Georgia with different management (Franzluebbers and Stuedemann, 2008). The closest associations were between cumulative C mineralization during 24 d of incubation and either basal soil respiration or the flush of CO<sub>2</sub>. Particulate organic C had the lowest association with other fractions but was still highly significant. Soil organic N fractions were also mostly strongly associated with each other (Table 4). Net N mineralization was most closely associated with TSN and least with particulate organic N. Across the 57 fields, TOC/TSN ratio was  $12.1 \pm 1.6 \text{ g s}^{-1}$ , particulate organic C/N ratio was  $19.4 \pm 3.2 \text{ g s}^{-1}$ , and mineralizable C/N ratio was 9.2  $\pm$  3.2 g g<sup>-1</sup>. The ratio of particulate to TOC was 172  $\pm$ 48 mg g<sup>-1</sup> TOC, and the ratio of particulate to total soil N was  $110 \pm 39 \text{ mg g}^{-1}$  TSN. As a fraction of TOC, soil microbial biomass C was 51.1  $\pm$  7.9 mg g<sup>-1</sup> TOC, cumulative C mineralization was 39.1  $\pm$  8.5 mg  $\rm CO_2-C~g^{-1}~TOC~24~d^{-1},$  and basal soil respiration was  $0.97 \pm 0.24$  mg CO<sub>2</sub>-C g<sup>-1</sup> TOC d<sup>-1</sup>. As a fraction of TSN, net N mineralization was  $53.3 \pm 12.0$  mg inorganic N  $g^{-1}$  TSN 24  $d^{-1}$ . The ratio of the flush of CO<sub>2</sub> to net N mineralization was  $3.3 \pm 0.9$  g g<sup>-1</sup>.

<sup>‡</sup> pH = -log [H<sup>+</sup>].

Table 4. Correlations among soil N fractions at depth of 0 to 10 cm across 57 field trials on tall fescue pasture.

Variable†	TSN	PON	RIN	RSN	RSA	NMIN
TSN	-	0.54	0.84	0.70	0.61	0.73
PON	***	-	0.58	0.48	0.52	0.43
RIN	***	**	-	0.84	0.80	0.83
RSN	***	**	***	-	0.94	0.96
RSA	***	NS‡	***	***	-	0.82
NMIN	***	***	***	***	***	-

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

<sup>†</sup> NMIN, net N mineralization (mg N kg<sup>-1</sup> soil 24 d<sup>-1</sup>); PON, particulate organic N (g N kg<sup>-1</sup> soil); RIN, residual inorganic N (mg N kg<sup>-1</sup> soil); RSA, residual soil ammonium (mg NH4-N kg<sup>-1</sup> soil); RSN, residual soil nitrate (mg NO3-N kg<sup>-1</sup> soil); TSN, total soil N (g N kg<sup>-1</sup> soil). <sup>‡</sup> Not significant (p > 0.05).

Net N mineralization during 24 d of incubation varied widely among fields. Median net N mineralization among fields was 122 mg N kg<sup>-1</sup> 24 d<sup>-1</sup>, with the middle 50% of data distributed in a range of 83 to 157 mg N kg<sup>-1</sup> 24 d<sup>-1</sup>. Calculated on an area basis with consideration of bulk density, potential net N mineralization in the field would have been  $136 \pm 50$  kg N ha<sup>-1</sup> among fields (depth of 0–10 cm). Residual inorganic N was  $17 \pm$ 9 kg N ha<sup>-1</sup>. Plant-available N (summation of residual inorganic N and net N mineralization) was  $153 \pm 57$  kg N ha<sup>-1</sup>, which is a considerable amount of N potentially available for plant uptake. Of the total N assumed potentially available for forage uptake, only  $11 \pm 4\%$  was originally in the inorganic form at the beginning of the fall stockpile period. This calculation is based on restricted soil sampling depth (0-10 cm) and assumed translation of net N mineralization in standard laboratory conditions to field conditions. The 24-d incubation period at 25°C and 50% water-filled pore space can be scaled to actual conditions in the field, and likely the time required to achieve these standard conditions will be several orders longer (e.g., achieving equivalent cumulative conditions might require ~100 d in the field with suboptimal temperature and moisture).

#### **Forage Yield Characteristics**

Forage DM production in the  $\geq 10$ -cm layer averaged 1388 kg ha<sup>-1</sup> across 20 field trials in 2015 and 1090 kg ha<sup>-1</sup> across 35 field trials in 2016 (Table 5). In the 5- to 10-cm forage layer in 2016, an additional 1597 kg ha<sup>-1</sup> DM was harvested. In 2016, total forage production ( $\geq 5$  cm height) averaged 2687 kg ha<sup>-1</sup>. Dry matter generally increased with increasing N fertilizer rate, although with diminishing effect toward higher N rate.

Forage moisture at the time of harvest in the beginning of winter was 616 mg g<sup>-1</sup> in the ≥10-cm forage layer in 2015 and 575 mg g<sup>-1</sup> in 2016 (Table 5). In the 5- to 10-cm forage layer in 2016, moisture averaged 559 mg g<sup>-1</sup>. Forage moisture averaged 569 mg g<sup>-1</sup> in the total harvest (≥5 cm height) in 2016. In all sets, forage moisture increased with increasing N fertilizer rate, likely because forage growth was more active later in the season with greater N availability.

Carbon concentration in forage averaged 447 mg  $g^{-1}$  in 2015 and 460 mg  $g^{-1}$  in the 10-cm layer, 454 mg  $g^{-1}$  in the 5- to 10-cm layer, and 457 mg  $g^{-1}$  in the total harvest in 2016 (Table 5). Carbon concentration was not affected by N fertilizer rate. Because of the relatively static C concentration, forage C uptake was essentially influenced only by DM production. Forage C

Table 5. Forage yield characteristics by year and forage layer and as affected by N fertilizer rate across field trials (n = 20 in 2015 and n = 35 in 2016).

	2015		2016	
N fertilizer	>10-cm layer	>10-cm layer	5- to 10-cm	Total
kg N ha <sup>-I</sup>			layer	(>5-cm layer)
Forage dry ma	tter (DM) prod	uction (kg DM	ha <sup>-1</sup> )	
0	1193	992	1538	2531
45	1386	1060	1603	2663
90	1443	1148	1634	2782
134	1528	1159	1615	2774
CV (%)	18.2	20.7	13.3	14.5
Forage moistu	re (mg water g <sup>-</sup>	<sup>-1</sup> field-moist fo	rage)	
0	597	547	534	542
45	611	572	558	567
90	625	582	566	576
134	633	599	578	589
CV (%)	3.8	5.7	6.4	5.6
Forage C cond	entration (mg 0	C g <sup>-1</sup> DM)		
0	446	460	455	457
45	447	460	455	457
90	446	460	454	456
134	447	459	454	456
CV (%)	0.6	0.8	1.2	0.9
Forage C upta	ke (kg C ha <sup>-1</sup> )			
0	530	457	701	1157
45	618	488	729	1217
90	642	528	742	1270
134	681	532	734	1266
CV (%)	18.0	20.7	13.3	14.5
Forage N cond	entration (mg l	Ng <sup>−I</sup> DM)		
0	19.9	17.6	17.4	17.5
45	21.6	19.7	19.0	19.3
90	23.1	21.2	20.1	20.6
134	24.2	22.9	21.3	22.0
CV (%)	7.3	7.2	6.1	6.0
Forage N upta	ke (kg N ha <sup>-1</sup> )			
0	23.8	17.3	26.6	43.9
45	29.5	20.7	30.1	50.8
90	32.5	24.0	32.6	56.7
134	36.1	26.2	33.9	60.2
CV (%)	18.4	21.5	14.1	15.3
Forage C/N ra	tio (g C g <sup>-1</sup> N)			
0	23.3	27.4	27.2	27.2
45	21.3	24.2	24.7	24.4
90	19.8	22.4	23.0	22.7
134	18.8	20.6	21.8	21.2
CV (%)	7.1	9.2	6.6	7.0

uptake averaged 618 kg ha<sup>-1</sup> in 2015 at the  $\geq$ 10-cm cutting height and 501 kg ha<sup>-1</sup> in the  $\geq$ 10-cm layer, 727 kg ha<sup>-1</sup> in the 5- to 10-cm layer, and 1228 kg ha<sup>-1</sup> in the total harvest in 2016. Forage C uptake generally increased with increasing N fertilizer rate, although with diminishing effect toward higher N rate like that of DM production.

Nitrogen concentration in forage averaged 22.2 mg g<sup>-1</sup> in 2015 and 20.4 mg g<sup>-1</sup> in the  $\geq 10$ -cm layer, 19.4 mg g<sup>-1</sup> in the 5- to 10-cm layer, and 19.8 mg g<sup>-1</sup> in the total harvest ( $\geq$ 5-cm height) in 2016 (Table 5). Increasing N fertilizer rate led to progressively greater forage N concentration with no evidence of

saturation. Therefore, forage N uptake was influenced by both N concentration and DM production. Forage N uptake averaged 31 kg N ha<sup>-1</sup> in the  $\geq$ 10-cm layer in 2015 and 22 kg N ha<sup>-1</sup> in the  $\geq$ 10-cm layer, 31 kg N ha<sup>-1</sup> in the 5- to 10-cm layer, and 53 kg N ha<sup>-1</sup> in the total harvest in 2016. In all sets, forage N uptake increased with increasing N fertilizer rate.

Across years, forage N concentration in the  $\geq 10$ -cm layer was 18.3  $\pm$  3.7 mg N g<sup>-1</sup> forage without N fertilizer and 23.1  $\pm$ 3.3 mg N g<sup>-1</sup> forage with sufficient N fertilizer to achieve maximum yield. Forage N concentration without N fertilizer was equivalent to 11.4  $\pm$  2.3% protein and with N fertilizer was 14.4  $\pm$  2.1% protein, the range of which is typically encompassed in other fall stockpile studies (Poore and Drewnoski, 2010). In the 5- to 10-cm forage layer, forage N concentration was 17.5  $\pm$ 3.5 mg N g<sup>-1</sup> forage without N fertilizer to achieve maximum yield.

Forage C/N ratio averaged 20.8 g g<sup>-1</sup> in the  $\geq 10$ -cm layer in 2015 and 23.7 g g<sup>-1</sup> in the  $\geq 10$ -cm layer, 24.2 g g<sup>-1</sup> in the 5- to 10-cm layer, and 23.9 g g<sup>-1</sup> in the total harvest in 2016 (Table 5). Forage C/N ratio always declined with increasing N fertilizer rate as a result of rising N concentration and static C concentration.

The CVs were generally similar in the  $\geq$ 10-cm forage layer between 2015 and 2016, so combining data across years was considered appropriate (Table 5). The CV was greatest for forage DM production and C and N uptake (>10%) and lowest for C concentration of forage tissue (~1%). For many forage characteristics, the CV was slightly lower in the 5- to 10-cm layer than in the  $\geq$ 10-cm layer. Reduced variation in the layer closest to the ground was likely due to the typically robust forage stands of tall fescue with vigorous tiller production in the fall, whereas variable top growth was dependent on leaf elongation.

#### Forage Yield Response to N Fertilizer Application

Yield response to N fertilization varied considerably among the 55 field trials (Table 6). Three examples of DM response to N fertilizer are shown in Fig. 5 to illustrate the types and extent of responses encountered. For DM production in the ≥10-cm layer, 22 of the trials had a nonlinear response, 24 of the trials had a linear response, and 9 of the trials had no response (Table 6). Yield without N fertilizer in the ≥10-cm layer was  $1058 \pm 497$  kg ha<sup>-1</sup>. Forage N uptake in this layer without N fertilizer applied was  $19 \pm 11 \text{ kg N} \text{ ha}^{-1}$ . Maximum DM yield in the ≥10-cm layer among fields was 1306 ± 528 kg ha<sup>-1</sup>. Forage N uptake in this layer at maximum yield was  $30 \pm 12$  kg N ha<sup>-1</sup>. Relative yield without N fertilizer compared with that with maximum yield at  $134 \text{ kg N} \text{ ha}^{-1} \text{ was } 0.82 \pm 0.16 \text{ g g}^{-1}$ . Of the 55 trials, 18 achieved at least 90% of maximum yield without N fertilizer applied. Therefore, a significant number of trials had little to no yield response (≥10-cm layer) to N fertilizer.

The lack of yield response to N fertilizer input would appear at odds with the literature because many studies have reported significant forage DM increases with N fertilizer application to tall fescue (Balasko, 1977; Collins and Balasko, 1981; Cogger et al., 2001; Wolf and Opitz von Boberfeld, 2003). On newly established tall fescue in Kentucky, instantaneous yield response to N fertilizer application was 45 kg forage DM kg<sup>-1</sup> N across 3 yr of evaluation (stockpiled mid-August to 1 December) (Taylor and Templeton, 1976). From a variety of tall fescue cultivars cut three to four times per year over 3 yr in Kentucky, instantaneous yield response to N fertilizer application had a median of 22 kg forage DM kg<sup>-1</sup> N, and the middle 50% of data from 15 trials had values of 13 to 25 kg forage DM kg<sup>-1</sup> N (Collins, 1991). In an evaluation of fall-stockpiled tall fescue in central Missouri, instantaneous yield response to N fertilizer application was 13 to 22 kg forage DM kg<sup>-1</sup> N among 3 yr (Gerrish et al., 1994). However, a recent study reported significant DM production without N fertilizer and minimal DM response to added N fertilizer (Teutsch et al., 2005) as well as widely variable responses depending on weather conditions and overall modest DM response to N fertilizer application on a long-term tall fescue pasture (Teutsch et al., 2011). It is possible that other studies have been conducted with little to no yield response to N fertilizer application and simply not published; as such, "negative results" are often not as highly regarded and readily explainable (Goodchild van Hilten, 2015).

Economically optimum N fertilizer requirement at a low costto-value threshold (i.e., 5 kg DM kg<sup>-1</sup> N) for forage production in the  $\geq$ 10-cm layer ranged from 0 to 134 kg N ha<sup>-1</sup> (maximum rate tested). Nine of the trials (16%) had EONR between 0 and 45 kg N ha<sup>-1</sup>, whereas only 9% of the trials had EONR >45 kg N ha<sup>-1</sup> (5% with EONR between 45 and 90 kg N ha<sup>-1</sup> and 4% with EONR between 90 and 134 kg N ha<sup>-1</sup>). The majority of trials (75%) had EONR of 0 for forage production in the  $\geq$ 10-cm layer. Ten of the trials had severe drought, so they would not have been expected to respond to N fertilizer (Teutsch et al., 2011). Analyses that follow are without those 10 droughtaffected trials unless otherwise noted.

Additional forage DM produced with N fertilizer was weakly but significantly negatively associated with plantavailable N (Fig. 6). We expected that the size of the readily available pool of N would help satisfy the need for N in these pasture systems, and it did, but not as strongly as anticipated. The lowest plant-available N was 64 kg N ha<sup>-1</sup>; therefore, sufficiently infertile sites were perhaps not present to drive this relationship to greater strength. It has been well established that accumulation of soil organic C and N occurs with longterm pasture development (Franzluebbers and Stuedemann, 2010) and that this organic matter accumulation leads to a significant and steady increase in mineralizable N in surface soil (Franzluebbers and Stuedemann, 2001; Franzluebbers et al., 1999). What is not clear is at what level of organic matter accumulation does a shift occur from net N immobilization to net N mineralization (Franzluebbers, 1999).

Plant-available N (i.e., residual inorganic + mineralizable) was considered the most accurate estimate of N availability. However, because soil-test biological activity (i.e., the flush of  $CO_2$  during 3 d) was highly associated with plant-available N (Fig. 7), we explored how well it fit with yield responses to N fertilizer. The flush of  $CO_2$  is considered an easily determined and more rapid soil biological indicator than mineralizable N and residual inorganic N. Several previous studies have also documented the close relationship between net N mineralization and the flush of  $CO_2$  (Franzluebbers, 1999; Franzluebbers and Stuedemann, 2008; Franzluebbers et al., 2018).

For DM production in the  $\geq 10$ -cm forage layer, values of EONR on an absolute basis (kg N ha<sup>-1</sup>) were most strongly associated with soil-test biological activity (r = -0.37; p = 0.01) among all soil variables measured or calculated. Other soil

Table 6. Forage dry matter and N i	uptake regression parame	ters in the ≥10-cm forage	layer of 55 fall-stoc	kpiled field trials
------------------------------------	--------------------------	---------------------------	-----------------------	---------------------

State/county/location code			Fora	age dry n	natter					For	age N up	otake		
	Model†	$DM_0^{\ddagger}$	а	b	RMSE	$DM_{max}$	EONR	Model	N <sub>0</sub>	а	b	RMSE	N <sub>max</sub>	EONR
				kg	ha <sup>-1</sup> —						kg	N ha <sup>-1</sup> –		
Coastal Plain region														
NC Johnston (104-CCRS)	L	1529	Ι	1.3	144	1701	0	NL	27.8	11.6	0.021	3.1	38.7	42
NC Pender (033-LBZZ)	L	980	I	0.6	277	1060	0	L	20.9	I	0.06	5.0	28.4	0
NC Wayne (034-CRFS)	L	550	I	1.4	73	735	0	L	10.4	I	0.05	1.8	17.7	0
NC Wayne (035-CRFD)	М	1916	0	0	236	1916	0	NL	35.7	12.6	0.013	5.7	46.2	39
NC Wayne (105-CRFZ)	NL	1387	212	0.051	213	1598	15	NL	21.4	23.1	0.007	3.4	34.9	62
Coastal Plain mean	-	1272	-	-	189	1402	3	-	23.2	-	-	3.8	33.2	29
Piedmont region														
GA Oconee (016-UGAZ)§	М	1580	0	0	126	1580	0	L	28.3	I	0.06	3.6	36.8	0
GA Oglethorpe (017-HTZZ)§	L	507	Ι	0.2	138	537	0	NL	8.5	6.2	0.038	2.3	14.7	23
GA Oglethorpe (018-WFZZ)§	L	313	Ι	0.1	80	325	0	L	8.5	I	0.01	2.3	10.1	0
GA Wilkes (019-WHZZ)§	L	325	Ι	0.1	115	341	0	NL	4.6	2.2	0.060	1.5	6.8	5
NC Durham (031-BCCZ)	NL	619	1104	0.005	284	1166	23	NL	8.2	60.0	0.002	5.0	22.5	97
NC Durham (102-BCC1)	L	1560	Ι	2.1	309	1842	0	NL	30. I	15.1	0.019	7.0	44.2	56
NC Durham (103-BCCN)	NL	729	1696	0.011	235	2019	121	NL	9.8	40.2	0.007	4.I	34.7	134
NC Granville (032-LDZZ)	L	1080	Ι	3.4	191	1543	0	L	17.3	I	0.11	3.5	32.5	134
NC Granville (113-LDZZ)	NL	1541	546	0.038	379	2084	37	NL	26.8	21.5	0.014	8.6	45.I	79
NC Guilford (027-YDZZ)	L	844	Ι	0.2	108	874	0	L	15.2	Ι	0.03	1.6	19.3	0
NC Montgomery (107-SRSZ)	NL	472	154	0.029	103	622	0	NL	9.7	8.3	0.017	2.4	17.2	21
NC Person (030-RJZZ)	NL	1218	439	0.042	221	1656	31	NL	18.1	19.3	0.016	4.2	35.3	70
NC Person (116-RJZZ)	NL	1400	812	0.008	115	1923	29	NL	28.7	28.5	0.005	3.0	42.7	72
NC Randolph (026-PRZZ)	L	1077	Ι	2.4	173	1395	0	L	19.4	I	0.08	3.6	30.2	0
NC Rockingham (028-BJZZ)	М	506	0	0	94	506	0	NL	8.7	2.6	0.011	1.9	10.7	0
NC Rockingham (029-UPRS)	NL	1215	144	0.063	198	1359	9	NL	25.9	8.8	0.018	4.3	33.9	26
NC Rockingham (114-UPRM)	NL	919	671	0.016	116	1511	48	NL	20.2	20.4	0.012	3.4	36.5	75
NC Rockingham (115-UPRG)	М	2127	0	0	140	2127	0	L	43.I	I	0.05	3.6	49.5	0
NC Rowan (020-PRSN)	NL	507	101	0.030	63	606	0	NL	7.6	9.3	0.007	1.3	13.1	0
NC Rowan (021-PRSO)	L	605	T	5.2	119	1305	134	L	7.3	I	0.14	2.5	25.7	134
NC Rowan (117-PRSL)	L	2240	T	1.6	340	2455	0	L	61.4	Ι	0.06	7.0	70.1	0
NC Rowan (118-PRSU)	NL	656	1143	0.009	143	1435	78	NL	12.0	52.0	0.004	3.9	31.9	134
NC Stanly (025-LNZZ)	М	2027	0	0	239	2027	0	L	39.8	I	0.03	5.6	43.7	0
NC Surry (022-JMRE)§	NL	862	171	0.039	144	1032	7	NL	12.2	7.7	0.018	3.0	19.3	19
NC Surry (023-JMBO)§	L	1375	I	1.4	470	1558	0	NL	21.7	11.5	0.011	8.7	30.6	20
NC Surry (024-JMBH)§	NL	1355	409	0.010	171	1659	0	NL	22.5	33.2	0.004	3.6	36.6	76
NC Surry (108-JMLF)	NL	1144	1777	0.002	152	1494	0	NL	22.0	17.2	0.007	2.9	32.3	23
NC Surry (109-JMRW)	NL	845	408	0.023	400	1234	27	NL	14.6	14.1	0.015	9.1	26.8	50
NC Wake (121-LWRZ)	L	1644	Ι	1.1	182	1787	0	L	29.3	Ι	0.09	3.4	41.0	0
VA Culpeper (008-WFZZ)§	L	874	Ι	1.0	174	1006	0	L	14.8	Ι	0.04	3.2	19.6	0
VA Fauquier (007-RFZZ)	NL	1153	696	0.013	304	1726	46	L	19.9	Ι	0.16	7.1	40.7	134
VA Goochland (011-NRZZ)	L	1913	Ι	0.3	137	1949	0	NL	34.5	12.6	0.036	4.1	47.0	42
VA Halifax (012-MMZZ)	М	1263	0	0	173	1263	0	NL	23.0	3.9	0.182	2.5	26.9	П
VA Loudoun (101-LCZZ)	NL	1609	473	0.020	310	2065	32	NL	24.0	42.1	0.004	5.1	45.6	138
Piedmont mean	_	1121	-	_	195	1412	18	_	20.5	-	_	4.1	31.6	46
Ridge and Valley region														
NC Ashe (013-UMRS)	М	1824	0	0	516	1824	0	NL	32.5	3.0	0.019	10.5	35.3	0
NC Ashe (III-UMRG)	L	545	Т	0.5	129	623	0	L	11.6	Ι	0.04	3.0	16.7	0
NC Ashe (112-UMRW)	м	1009	0	0	163	1009	0	NL	20.1	4.4	0.020	3.7	24.2	0
NC Clay (015-HBZZ)6	L	769	I	4.2	255	1328	0	L	11.0	Ì	0.12	4.9	27.4	134
NC Clay (106-HBZZ)	L	867	I	1.3	213	1043	0	L	21.9	I	0.05	5.2	29.0	0
NC Haywood (014-MRSZ)	м	316	0	0	163	316	0	м	6.7	0	0	3.6	6.7	0
NC Haywood (119-MRSL)	L	829	I	1.3	211	999	0	L	20.0	Ī	0.08	6.4	30.6	0
NC Haywood (120-MRSU)	NL	824	96	0.028	113	918	0	NL	19.7	7.2	0.013	3.3	25.7	0
VA Augusta (009-SVAI)	L	1182	I	2.1	150	1461	0	L	18.3		0.11	2.7	32.5	134
<b>U</b> (1111)		-		-		-	-							

Continued

State/county/location code	Forage dry matter								Forage N uptake					
	Model†	DM <sub>0</sub> ‡	а	b	RMSE	DM <sub>max</sub>	EONR	Model	N <sub>0</sub>	а	b	RMSE	N <sub>max</sub>	EONR
VA Augusta (010-SVAU)	L	1274	Ι	2.3	194	1579	0	L	21.8	I	0.11	5.2	36.1	134
VA Carroll (004-SMR1)	NL	547	699	0.007	225	958	0	NL	11.7	73.6	0.001	5.2	25.0	57
VA Carroll (005-SMHP)	NL	1064	69	0.021	193	1129	0	NL	22.3	9.4	0.013	5.4	30.0	13
VA Carroll (006-SMVVL)	NL	298	246	0.016	88	515	0	NL	7.0	8.2	0.014	2.0	14.0	П
VA Pulaski (003-SBZZ)§	L	895	I.	3.0	119	1304	0	L	15.6	I.	0.12	2.8	31.4	134
WV Monongalia (001-KFIM)	L	699	I.	1.4	89	894	0	NL	11.4	15.6	0.007	2.1	21.2	19
WV Monongalia (002-KFUN)	NL	787	173	0.007	122	889	0	NL	6.0	14.7	0.004	2.0	11.6	0
Ridge and Valley mean	-	858	-	-	184	1049	0	-	16.1	-	-	4.2	24.8	40

+ L, linear (Y = Y<sub>0</sub> + b · X); M, mean (Y = Y<sub>0</sub>); NL, nonlinear [Y = Y<sub>0</sub> + a · e<sup>(-b · X)</sup>], where Y<sub>0</sub> = DM<sub>0</sub> for dry matter and N<sub>0</sub> for N uptake.

 $\pm$  DM<sub>max</sub>, maximum dry matter from regression at 134 kg N ha<sup>-1</sup> rate; EONR, economically optimum N fertilizer requirement; N<sub>max</sub>, maximum N uptake from regression at 134 kg N ha<sup>-1</sup> fertilizer rate.

§ Drought-affected field.



Fig. 5. Examples of yield responses to N fertilizer application at three field trials. Triangle,  $\geq$ 10-cm layer; square, 5- to 10-cm layer. Solid line is best-fit regression for  $\geq$ 10-cm layer. Dashed line is best-fit regression for 5- to 10-cm layer.

biological properties that were significantly associated with EONR included plant-available N (r = -0.35; p = 0.02), net N mineralization during 24 d (r = -0.33; p = 0.03), and cumulative C mineralization (r = -0.32; p = 0.04). Extractable P was positively associated with EONR (r = 0.31; p = 0.04), suggesting that sites with greater extractable P had greater likelihood of response to N fertilizer. No other soil properties were significantly associated with EONR in the ≥10-cm forage layer. Expressed as EONR per ton of forage, all of the same variables were similarly influential in the ≥10-cm forage layer. In addition, CEC was positively associated with EONR (r = 0.23; p = 0.05). The weak positive associations of extractable P and CEC with EONR are not readily explainable, so the interaction of N availability with other indicators of fertility (i.e., P and CEC) on fall stockpile response to N fertilizer application deserves attention.



Fig. 6. Additional forage dry matter (DM) produced beyond that without N fertilizer as affected by plant available N (PAN)across 45 field trials.



Fig. 7. Association of plant-available N (PAN) (residual inorganic + mineralizable during 24 d) with soil-test biological activity (STBA) as measured by the flush of  $CO_2$  during 3 d across 56 field trials. One trial was omitted from the regression (gray-filled square) because it did not coincide with other trials when compared with net N mineralization.

Figure 8 shows how soil-test biological activity was associated in a nonlinear manner with EONR at a low cost-to-value threshold when expressed on an absolute basis (kg N ha<sup>-1</sup>) and per ton of forage (kg N Mg<sup>-1</sup> forage). Many fields selected for this evaluation had sufficiently high soil-test biological activity, which appears to



Fig. 8. Association of economically optimum N fertilizer requirement (EONR) on (top) absolute basis and (bottom) per ton of forage produced in the  $\geq$ 10-cm forage layer with soil-test biological activity (STBA) as determined by the flush of CO<sub>2</sub> during 3 d. Forty-five field trials (excluding 10 drought-affected trials) were sorted by ascending order of soil-test biological activity. Means were calculated for five consecutive trials to reduce variation in raw data.

have been a key indicator of whether forage yield response to N fertilizer application would occur. Based on these relationships, a key inflection point that led to drastically diminished yield response to N fertilizer was ~500 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil 3 d<sup>-1</sup>. In fact, if the data in Fig. 8 were fitted to a linear relationship, EONR = 0 would have been reached at 540 mg  $CO_2$  – C kg<sup>-1</sup> soil 3 d<sup>-1</sup> in both cases. Data from five neighboring fields along the soil-test biological activity gradient were pooled to clarify the relationship because there was a large amount of variation among sites. This variation can be expected from the wide range of soil types, environmental conditions, and management that were explored (Table 1), as well as the diversity of regression models selected and how these regressions incorporate relatively large RMSE from field response data into a single estimate of EONR for each field trial (Table 6). As a case in point, meeting the cost-to-value threshold of 5 kg DM kg<sup>-1</sup> N was narrowly met for Location 021-PRSO in Rowan County, NC (parameters a  $\times$  b = 5.2), and, because the best model was a linear fit, the EONR was calculated as the highest rate test (i.e., 134 kg N ha<sup>-1</sup>). With slight curvature in the response or slightly lower linear estimate, EONR could have been 0 kg N ha<sup>-1</sup>. The dilemma of obtaining accurate estimates of fertilizer N recommendations from yield response curves is not unique to forage management systems (Morris et al., 2018).

In the second year of evaluation, we harvested forage at two layers to assess whether a significant yield response to N fertilizer



Fig. 9. Association of economically optimum N fertilizer requirement (EONR) on (top) absolute basis and (bottom) per ton of forage produced for  $\geq$ 10-cm forage layer, 5- to 10cm forage layer, and total forage ( $\geq$ 5-cm layer) with soil-test biological activity (STBA) as determined by the flush of CO<sub>2</sub> during 3 d. Twenty-five field trials (excluding 10 drought-affected trials) were sorted by ascending order of soil-test biological activity. Means were calculated for five consecutive trials to reduce variation in raw data. Regression equation in each panel is for total forage only ( $\geq$ 5 cm layer).

occurred closer to the soil surface. There were 25 field trials that could be evaluated (10 drought-affected trials were deleted from these analyses). Forage DM in the  $\geq$ 10-cm layer was 42 ± 6% of total DM harvested at the  $\geq$ 5-cm height. Therefore, there was considerable forage present below the 10-cm height. However, as seen from data in Fig. 9, DM response to N fertilizer was limited to sites with relatively low soil-test biological activity, and the effect was considerably lower than that expressed in the ≥10-cm forage layer. However, when the two forage layers were summed, the association between EONR at low cost-to-value threshold with soil-test biological activity was stronger than in the ≥10-cm forage layer only. These data show clearly that soil-test biological activity could be a useful indicator of soil N availability and could be developed further to help guide actual N fertilizer recommendations for fall-stockpiled tall fescue. The data also clearly show that many tall fescue pastures have sufficiently high soil-test biological activity, precluding the benefit of N fertilizer to enhance DM production. The value of 20 kg N Mg<sup>-1</sup> forage DM in Fig. 9 relates reasonably well to general N fertilizer recommendations in the region, wherein a forage yield expectation of 3 Mg DM ha<sup>-1</sup> would receive 60 kg N ha<sup>-1</sup> and a forage yield expectation of 5 Mg DM ha<sup>-1</sup> would receive 100 kg N ha<sup>-1</sup>. Such a recommendation system implies that all sites

Table 7. Forage dry matte	er and nitrogen uptake	regression parameters	in the total forage	e harvested (≥5	cm height) from	35 fall-stockpiled
field trials in 2016.			_			

	Forage dry matter					Forage N uptake								
State/county/location code	Model†	DM <sub>0</sub> ‡	а	b	RMSE	DM <sub>max</sub>	EONR	Model	N <sub>0</sub>	а	b	RMSE	N <sub>max</sub>	EONR
					a <sup>-1</sup> —				•		— kg N	ha <sup>-1</sup> ——	max	
Coastal Plain region														
NC Pender (033-LBZZ)	L	2671	I	1.1	453	2820	0	NL	49.6	17.5	0.086	5.4	67.I	31
NC Wayne (034-CRFS)	NL	1593	385	0.009	185	1869	0	L	28.4	Ι	0.10	3.5	42.I	134
NC Wayne (035-CRFD)	NL	4474	416	0.035	490	4886	30	NL	83.5	33.6	0.016	10.6	112.9	107
Coastal Plain mean	-	2913	-	-	376	3191	10	-	53.8	-	-	6.5	74.0	91
Piedmont region														
GA Oconee (016-UGAZ)§	Μ	355 I	0	0	192	3551	0	NL	63.7	24.4	0.006	6.5	76.9	61
GA Oglethorpe (017-HTZZ)§	Μ	1563	0	0	331	1563	0	NL	27.6	11.1	0.036	5.8	38.6	38
GA Oglethorpe (018-WFZZ)§	Μ	1249	0	0	207	1249	0	L	32.2	I	0.02	5.0	34.2	0
GA Wilkes (019-WHZZ)§	Μ	1461	0	0	304	1461	0	NL	22.0	4.7	0.083	3.3	26.7	16
NC Durham (031-BCCZ)	L	2224	I.	5.4	491	2954	134	L	30.0	I	0.19	7.7	55.0	134
NC Granville (032-LDZZ)	L	3260	I.	6.3	377	4102	134	L	54. I	Ι	0.23	6.5	84.3	134
NC Guilford (027-YDZZ)	NL	2102	256	0.023	310	2346	7	NL	38.5	14.6	0.010	5.4	49.5	40
NC Person (030-RJZZ)	L	3070	I	3.0	484	3468	0	NL	43.I	27.2	0.016	8.2	67.3	91
NC Randolph (026-PRZZ)	NL	2613	3813	0.001	290	3264	44	NL	49.2	47.0	0.004	5.8	69.6	134
NC Rockingham (028-BJZZ)	Μ	2038	0	0	255	2038	0	NL	36.6	10.6	0.011	5.2	44.7	12
NC Rockingham (029-UPRS)	NL	3104	205	0.040	189	3308	13	NL	63.7	17.1	0.013	3.5	77.9	61
NC Rowan (020-PRSN)	L	1441	I.	1.3	139	1613	0	NL	20.9	19.2	0.006	2.1	32.0	31
NC Rowan (021-PRSO)	L	1630	I	6.5	189	2498	134	L	19.3	Ι	0.21	3.6	46.9	134
NC Stanly (025-LNZZ)	Μ	4395	0	0	357	4395	0	L	86.9	Ι	0.04	8.3	92.6	0
NC Surry (022-JMRE)§	L	2626	I	1.3	391	2800	0	NL	40.2	19.9	0.010	8.3	55.2	70
NC Surry (023-JMBO)§	L	3308	I	0.2	687	3331	0	L	57.9	Ι	0.07	13.6	67.7	0
NC Surry (024-JMBH)§	L	3721	I	2.2	344	4016	0	L	66.3	Ι	0.17	7.3	89.3	134
VA Culpeper (008-WFZZ)§	NL	2706	160	0.016	326	2846	0	NL	45.8	13.7	0.012	6.6	56.6	40
VA Fauquier (007-RFZZ)	NL	3044	975	0.017	516	3922	70	NL	50.6	93.6	0.003	13.3	85.0	134
VA Goochland (011-NRZZ)	L	3839	I	1.1	305	3992	0	NL	67.6	26.5	0.023	8.9	93.0	78
VA Halifax (012-MMZZ)	Μ	3216	0	0	282	3216	0	NL	56.2	7.9	0.042	4.3	64.I	28
Piedmont mean	-	2674	-	-	332	2949	26	-	46.3	-	-	6.6	62.2	65
Ridge and Valley region														
NC Ashe (013-UMRS)	Μ	3945	0	0	798	3945	0	NL	61.8	13.3	0.023	17.3	74.6	49
NC Clay (015-HBZZ) §	L	2375	I	6.2	460	3213	134	L	36.0	Ι	0.22	7.6	65.3	134
NC Haywood (014-MRSZ)	Μ	993	0	0	271	993	0	М	24.9	0	0	6.4	24.9	0
VA Augusta (009-SVAI)	L	2689	I.	3.8	221	3202	0	L	40.7	I	0.19	3.4	66.5	134
VA Augusta (010-SVAU)	L	3196	I.	2.3	242	3501	0	L	50.5	I	0.17	5.9	73.6	134
VA Carroll (004-SMRI)	NL	1314	855	0.010	315	1940	52	NL	27.3	82.3	0.002	5.9	48.4	134
VA Carroll (005-SMHP)	NL	2454	135	0.013	312	2564	0	NL	50.2	20.0	0.009	8.6	64.0	64
VA Carroll (006-SMVVL)	NL	1121	257	0.017	179	1352	0	NL	25.6	10.1	0.021	3.9	35.0	36
VA Pulaski (003-SBZZ)§	NL	2103	791	0.009	222	2656	39	L	35.8	I	0.18	4.2	60.3	134
WV Monongalia (001-KFIM)	L	1529	Т	2.6	132	1873	0	NL	24.7	31.7	0.006	3.8	42.3	107
WV Monongalia (002-KFUN)	NL	1880	172	0.012	119	2020	0	NL	15.5	28.5	0.004	2.4	27.5	35
Ridge and Valley mean	-	2145	-	-	297	2478	21	-	35.7	-	_	6.3	52.9	88

 $\frac{1}{1}$  L, linear (Y = Y<sub>0</sub> + b · X); M, mean (Y = Y<sub>0</sub>); NL = nonlinear [Y = Y<sub>0</sub> + a · e<sup>(-b · X)</sup>], where Y<sub>0</sub> = DM<sub>0</sub> for dry matter and N<sub>0</sub> for N uptake.

<sup>‡</sup> DM<sub>max</sub>, maximum dry matter from regression at 134 kg N ha<sup>-1</sup> rate; EONR, economically optimum N fertilizer requirement (kg ha<sup>-1</sup>); N<sub>max</sub>, maximum N uptake from regression at 134 kg N ha<sup>-1</sup> fertilizer rate.

§ Drought-affected field.

have relatively modest soil-test biological activity, but this study showed this was not true.

Across 25 field trials with two forage layer harvests, efficiency of N fertilizer uptake in the  $\geq$ 5-cm forage layer was 0.27  $\pm$ 0.29 kg N uptake kg<sup>-1</sup> N applied at the instantaneous point of fertilizer application (i.e., product of parameters a and b in Table 7). The vast majority of N was, therefore, stored in crowns and roots, left in the soil as inorganic N, incorporated into soil organic matter, and/or lost from the soil system. To achieve at least 0.10 kg N uptake kg<sup>-1</sup> N applied in the  $\geq$ 5-cm layer, application of 79 ± 50 kg N ha<sup>-1</sup> was needed. There was no significant association of N fertilizer required to achieve a target recovery with soil-test biological activity. With inorganic and dairy manure application of N to tall fescue in British Columbia, apparent N recovery of applied N was 0.58 ± 0.08 kg N uptake kg<sup>-1</sup> N applied inorganically and 0.22 ± 0.06 kg N uptake kg<sup>-1</sup> N applied with manure (Bittman et al., 1999).

Efficiency of N utilization by tall fescue has been suggested in a similar manner when calculated as additional forage DM produced per unit of N applied, which Poore and Drewnoski (2010) summarized in a literature review as 7 to 33 kg DM kg<sup>-1</sup> applied N. The DM produced at the instantaneous point of fertilizer application (i.e., the product of parameters a and b in Tables 6 and 7) reflects this same approach. In the ≥10-cm forage layer, there were 41 trials that produced <5 kg DM kg<sup>-1</sup> N and 14 trials that produced  $\geq$ 5 kg DM kg<sup>-1</sup> N. Soil-test biological activity was 391  $\pm$  124 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil 3 d<sup>-1</sup> in the low-responding category and  $319 \pm 112 \text{ mg CO}_2 - \text{C kg}^{-1}$  soil  $3 \text{ d}^{-1}$  in the high-responding category (p = 0.03 between these categories of soil-test biological activity). In the  $\geq$ 5-cm forage layer in 2016 only, there were 24 trials that produced <5 kg DM kg<sup>-1</sup> N (associated with soil-test biological activity of  $402\pm127$  mg CO  $_2-C$  kg  $^{-1}$  soil 3 d  $^{-1})$  and 11 trials that produced  $\geq$ 5 kg DM kg<sup>-1</sup> N (associated with soiltest biological activity of  $332 \pm 121 \text{ mg CO}_2 - \text{C kg}^{-1} \text{ soil } 3 \text{ d}^{-1}$ ; p = 0.07 between these categories of soil-test biological activity). The greatest response in this study was 21 kg DM kg<sup>-1</sup> N in the ≥10-cm layer, which was only intermediate in the range reported by Poore and Drewnoski (2010). Our study appears to have sampled a wide range of field conditions, including those well endowed with biologically active soil N. In future research, a focus should be on clearly distinguishing infertile and fertile sites along as wide a gradient as possible but more so at the low end of fertility. The lowest soil-test biological activity measured in this study was 135 mg CO<sub>2</sub>–C kg<sup>-1</sup> soil 3 d<sup>-1</sup> on a sandy loam in Durham County, NC. We planned to target more low-fertility sites in the second year, but selected fields were still of moderate fertility. Soil-test biological activity is highly dependent on sampling depth (Franzluebbers and Stuedemann, 2015). However, values in cropland soils at the same depth increment can be <100 mg  $CO_2$ -C kg<sup>-1</sup> soil 3 d<sup>-1</sup>, especially in frequently tilled conditions (unpublished data).

#### CONCLUSIONS

Forage DM production, C and N uptake, and tissue N concentration and moisture were generally positively influenced by fertilizer N application at the end of summer prior to fall stockpiling of tall fescue stands. However, a diversity of responses occurred among 55 field trials during the 2 yr of evaluation. Relatively few trials had strong responses, several had intermediate/moderate response, and many had simply no response to N fertilizer application, depending on the variable of interest. The lack of low-fertility sites was a concern, and these conditions should be pursued in further research. Economically optimum N fertilizer requirement to achieve a low cost-to-value threshold of 5 kg DM kg<sup>-1</sup> N was 0 for 75% of the field trials, <45 kg N ha<sup>-1</sup> for 16% of the field trials, and >45 kg N ha<sup>-1</sup> for 9% of the field trials. Plant-available N (residual inorganic + mineralizable N) and soil biological activity were key indicators that helped discern among responsive and nonresponsive trials. The flush of  $CO_2$  is a soil biological property that has rapid analysis time, is relatively simple and inexpensive in methodological requirements, and relates to a diversity of other important soil C and N characteristics of biological origin. This evaluation supports the use of the flush of CO<sub>2</sub> as an appropriate indicator for soil-test biological activity. The strong association of the flush of CO<sub>2</sub> with plant-available N in this study across a diversity of soils in Georgia, North Carolina, Virginia, and West

Virginia supports the use of the flush of  $CO_2$  as a rapid and reliable indicator of soil N availability.

#### ACKNOWLEDGMENTS

Ellen Leonard, Ashtyn Mizelle, and Erin Silva provided sound technical support. Becky Barlow, Jonathan Black, Philip Brown, Rick Brown, Ben Chase, Mark Clements, J.B. Daniel, Brad Graham, Brad Hardison, Danny Hyatt, Sheryl Jarvis, Jay Marshall, Tim Mize, John Michael Phillips, Ed Rayburn, HR Scott, Robert Shoemaker, Lorie Townsend, and Jason Wheatley provided key logistical support at several field sites. Sincere appreciation is extended to all research station managers and collaborating farmers, including Dean Askew, Norman Bower, Jason Byrd, Eric Elsner, David Fiske, Joe French, Joe Hampton, Cathy Herring, Ross Holcomb, Ted Hughes, Bass Hyatt, Mike Jones, Rick Kern, William King, Buron Lanier, Bette and Doan Laursen, Carl Lindgren, Nathan Lowder, Jeremy Martin, Vance Mattox, Mike McDowell, Andy Meier, Ronnie Nuckols, Roger Pritchard, Kaleb Rathbone, Johnny Rogers, Blair Sanders, Greg Schaeffer, Mark Strauss, Carl Stafford, Tracy Taylor, Herman Wheatley, and Don York. Financial support was provided by USDA-ARS, USDA-Natural Resources Conservation Service Conservation Innovation Grant 69-3A75-14-251 and USDA-National Institute of Food and Agriculture, Competitive Grant Agreement No. 2013-67019-21369.

#### REFERENCES

- Balasko, J.A. 1977. Effects of N, P, and K fertilization on yield and quality of tall fescue forage in winter. Agron. J. 69:425–428. doi:10.2134/ agronj1977.00021962006900030023x
- Belesky, D.P., J.A. Stuedemann, R.D. Plattner, and S.R. Wilkinson. 1988. Ergopeptine alkaloid in grazed tall fescue. Agron. J. 80:209–212. doi:10.2134/agronj1988.00021962008000020014x
- Bittman, S., C.G. Kowalenko, D.E. Hunt, and O. Schmidt. 1999. Surface-banded and broadcast dairy manure effects on tall fescue yield and nitrogen uptake. Agron. J. 91:826–833. doi:10.2134/ agronj1999.915826x
- Brady, N.C., and R.R. Weil. 1999. The nature and properties of soils. 12th ed., Prentice Hall, Upper Saddle River, NJ.
- Buckner, R.C., J.B. Powell, and R.V. Frakes. 1979. Historical development. In: R.C. Buckner and L.P. Bush, editors, Tall fescue. Agron. Monogr. 20. ASA, CSSA, and SSSA, Madison, WI. p. 1–8. doi:10.2134/ agronmonogr20.c1
- Bundy, L.G., and J.J. Meisinger. 1994. Nitrogen availability indices. In: R.W. Weaver, J.S. Angle, and P.S. Bottomley, editors, Methods of soil analysis. Part 1. SSSA Book Ser. 5. SSSA, Madison, WI. p. 951–984.
- Castillo, M.S., J.C. Burns, D.A. Crouse, J.T. Green, D. Hardy, and D.L. Osmond. 2016. Forage fertilization in North Carolina: Concepts and guidelines. North Carolina State Extension. content.ces.ncsu. edu/forages-for-north-carolina-general-guidelines-and-concepts (accessed 1 Aug. 2018).
- Castillo, M.S., J.C. Burns, and S. Sosinski. 2018. Production and utilization of stockpiled tall fescue: Understanding the basic concepts. North Carolina State Extension. https://content.ces.ncsu.edu/ production-and-utilization-of-stockpiled-tall-fescue-understandingthe-basic-concepts (accessed 1 Aug. 2018).
- Cogger, C.G., A.I. Bary, S.C. Fransen, and D.M. Sullivan. 2001. Seven years of biosolids versus inorganic nitrogen applications to tall fescue. J. Environ. Qual. 30:2188–2194. doi:10.2134/jeq2001.2188
- Collins, M. 1991. Nitrogen effects on yield and forage quality of perennial ryegrass and tall fescue. Agron. J. 83:588–595. doi:10.2134/agronj19 91.00021962008300030017x

- Collins, M., and J.A. Balasko. 1981. Effects of N fertilization and cutting schedules on stockpiled tall fescue. I. Forage yield. Agron. J. 73:803– 807. doi:10.2134/agronj1981.00021962007300050014x
- Fribourg, H.H., and K.W. Bell. 1984. Yield and composition of tall fescue stockpiled for different periods. Agron. J. 76:929–934. doi:10.2134/ agronj1984.00021962007600060016x
- Franzluebbers, A.J. 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol. Biochem. 31:1083–1090. doi:10.1016/ S0038-0717(99)00022-X
- Franzluebbers, A.J. 2016. Should soil testing services measure soil biological activity? Agric. Environ. Lett. 1:150009. doi:10.2134/ ael2015.11.0009
- Franzluebbers, A.J., M.R. Pershing, C. Crozier, D. Osmond, and M. Schroeder-Moreno. 2018. Soil-test biological activity with the flush of CO<sub>2</sub>: I. C and N characteristics of soils in corn production. Soil Sci. Soc. Am. J. 82:685–695.
- Franzluebbers, A.J., N. Nazih, J.A. Stuedemann, J.J. Fuhrmann, H.H. Schomberg, and P.G. Hartel. 1999. Soil carbon and nitrogen pools under low- and high-endophyte-infected tall fescue. Soil Sci. Soc. Am. J. 63:1687–1694. doi:10.2136/sssaj1999.6361687x
- Franzluebbers, A.J., and J.A. Stuedemann. 2001. Bermudagrass management in the Southern Piedmont U.S. IV. Soil-surface nitrogen pools. Sci. World 1:673–681. doi:10.1100/tsw.2001.89
- Franzluebbers, A.J., and J.A. Stuedemann. 2008. Early response of soil organic fractions to tillage and integrated crop-livestock production. Soil Sci. Soc. Am. J. 72:613–625. doi:10.2136/sssaj2007.0121
- Franzluebbers, A.J., and J.A. Stuedemann. 2010. Surface soil changes during twelve years of pasture management in the Southern Piedmont USA. Soil Sci. Soc. Am. J. 74:2131–2141. doi:10.2136/ sssaj2010.0034
- Franzluebbers, A.J., and J.A. Stuedemann. 2015. Does grazing of cover crops impact biologically active soil C and N fractions under inversion and no tillage management? J. Soil Water Conserv. 70:365–373. doi:10.2489/jswc.70.6.365
- Franzluebbers, A.J., J.A. Stuedemann, and D.H. Franklin. 2012. Water infiltration and surface-soil structural properties as influenced by animal traffic in the Southern Piedmont USA. Renew. Agric. Food Syst. 27:256–265. doi:10.1017/S1742170511000378
- Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. In: A. Klute, editor, Methods of soil analysis. Part 1. Physical and mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI. p. 383–411.
- Gerrish, J.R., P.R. Peterson, C.A. Roberts, and J.R. Brown. 1994. Nitrogen fertilization of stockpiled tall fescue in the Midwestern USA. J. Prod. Agric. 7:98–104. doi:10.2134/jpa1994.0098
- Goodchild van Hilten, L. 2015. Why it's time to publish research "failures". www.elsevier.com/connect/scientists-we-want-your-negativeresults-too (accessed 1 Aug. 2018).
- Hancock, D.W., and R.W. Josey. 2008. Stockpiling tall fescue for fall and wintergrazing. University of Georgia Extension, Athens. http://extension.uga.edu/publications/detail.cfm?number=C920#Fertilizing (accessed 1 Aug. 2018).
- Johnson, G., and R. Smith. 2004. Stockpiling tall fescue. Virginia Cooperative Extension, Virginia Tech, Blackburg. www.sites.ext.vt.edu/ newsletter-archive/cses/2004-08/stockpiling.html (accessed 1 Aug. 2018).
- Kallenbach, R.L., G.J. Bishop-Hurley, M.D. Massie, G.E. Rottinghaus, and C.P. West. 2003. Herbage mass, nutritive value, and ergovaline concentration of stockpiled tall fescue. Crop Sci. 43:1001–1005. doi:10.2135/cropsci2003.1001

- Keeney, D.R., and J.M. Bremner. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J. 58:498–503. doi:10.2134/agronj1966.000219620058 00050013x
- Lemus, R. 2008. Extending the grazing season: Stockpiled tall fescue. Mississippi State Univ. Extension, Mississippi State. https://extension. msstate.edu/sites/default/files/publications/publications/p2461.pdf (accessed 1 Aug. 2018).
- Morris, T.F., T.S. Murrell, D.B. Beegle, J.J. Camberato, R.B. Ferguson, J. Grove, Q. Ketterings, P.M. Kyveryga, C.A.M. Laboski, J.M. McGrath, J.J. Meisinger, J. Melkonian, B.N. Moebius-Clune, E.D. Nafziger, D. Osmond, J.E. Sawyer, P.C. Scharf, W. Smith, J.T. Spargo, H. van Es, and H. Yang. 2018. Strengths and limitations of nitrogen rate recommendations for corn and opportunities for improvement. Agron. J. 110:1–37. doi:10.2134/agronj2017.02.0112
- Muruganandam, S., D.W. Israel, and W.P. Robarge. 2009. Activities of nitrogen-mineralization enzymes associated with soil aggregate size fractions of three tillage systems. Soil Sci. Soc. Am. J. 73:751–759. doi:10.2136/sssaj2008.0231
- Poore, M.H., and M.E. Drewnoski. 2010. Review: Utilization of stockpiled tall fescue in winter grazing systems for beef cattle. Prof. Anim. Sci. 26:142–149.
- Rayburn, E.B. 1993. Forage management: Tall fescue management. West Virginia Univ. Ext. Serv., Morgantown. https://extension.wvu.edu/ files/d/4335e33c-660e-445a-881f-433a86e02e72/tall-fescue-management-5184.pdf (accessed 1 Aug. 2018).
- Rottinghaus, G.E., G.B. Garner, C.N. Cronell, and J.L. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte-infected tall fescue: Seasonal variation of ergovaline levels in stem with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39:112– 115. doi:10.1021/jf00001a022
- Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. 36:465–472. doi:10.2136/ sssaj1972.03615995003600030029x
- Stuedemann, J.A., and C.S. Hoveland. 1988. Fescue endophyte: History and impact on animal agriculture. J. Prod. Agric. 1:39–44. doi:10.2134/jpa1988.0039
- Taylor, T.H., and W.C. Templeton, Jr. 1976. Stockpiling Kentucky bluegrass and tall fescue forage for winter pasturage. Agron. J. 68:235– 239. doi:10.2134/agronj1976.00021962006800020006x
- Teutsch, C.D., W.M. Clapham, J.M. Fedders, G. Groover, and W.M. Tilson. 2011. Nitrogen rate and application timing affect the yield and risk associated with stockpiling tall fescue for winter grazing. Forage Grazinglands 9(1). doi:10.1094/FG-2011-0513-01-RS
- Teutsch, C.D., J.H. Fike, G.E. Groover, and S. Aref. 2005. Nitrogen rate and source effects on the yield and nutritive value of tall fescue stockpiled for winter grazing. Forage Grazinglands 3(1). doi:10.1094/ FG-2005-1220-01-RS
- Voroney, R.P., and E.A. Paul. 1984. Determination of k<sub>C</sub> and k<sub>N</sub> in situ for calibration of the chloroform fumigation-incubation method. Soil Biol. Biochem. 16:9–14. doi:10.1016/0038-0717(84)90117-2
- Wang, Y., C. Tu, L. Cheng, C. Li, L.F. Gentry, G.D. Hoyt, X. Zhang, and S. Hu. 2011. Long-term impact of farming practices on soil organic carbon and nitrogen pools and microbial biomass and activity. Soil Tillage Res. 117:8–16. doi:10.1016/j.still.2011.08.002
- Wolf, D., and W. Opitz von Boberfeld. 2003. Effects of nitrogen fertilization and date of utilization on the quality and yield of tall fescue in winter. J. Agron. Crop Sci. 189:47–53. doi:10.1046/j.1439-037X.2003.00003.x