Soil Carbon and Nitrogen Pools in Response to Tall Fescue Endophyte Infection, Fertilization, and Cultivar

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ABSTRACT

Tall fescue (*Festuca arundinacea* Schreb.) is an important cool-season perennial forage used for grazing animals in the humid regions of the USA and throughout the world. The fungal endophyte *Neotyphodium coenophialum* Glenn, Bacon, & Hanlin naturally inhabits the majority of tall fescue stands producing a variety of alkaloids in leaf tissue that can cause animal health disorders on ingestion. We hypothesized that endophyte infection would modify the stock and activity of various soil C and N pools (total, particulate, microbial biomass, and mineralizable), but that fertilization (13.4–1.5–5.6 vs. 33.6–3.7–13.9 g N–P–K m^{-2} yr^{-1}) and cultivar ('Kentucky-31', K-31; 'AU-Triumph'; and 'Johnstone') might alter these responses. Soil organic C and total N at a depth of 0 to 20 cm under K-31 with high fertilization were greater with high (4197 g C m^{-2} and 266 g N m^{-2}) than with low (3872 g C m^{-2} and 242 g N m^{-2}) endophyte infection at the end of 20 yr. Under low fertilization, soil organic C and total N were not different between low and high endophyte infection. Differences in C and N pools among cultivars with low fertilization were as large as among K-31 fertilization-endophyte comparisons, but appeared to be related to factors other than endophyte infection frequency. Carbon and N contents of small macroaggregates (0.25–1.0 mm) were the only soil properties that were related (r = 0.70, P = 0.001) to endophyte infection frequency (range of 1–79%) across all treatments. Soil C and N pools can be modified by endophyte infection, but these results narrowed this phenomenon to (i) conditions of higher fertility and (ii) predominantly in small macroaggregates.

Tall fescue is the most important cool-season perennial forage in the eastern USA (Stuedemann and Hoveland, 1988), grown on approximately 14 Mha (Buckner et al., 1979). A natural, mutualistic association with a fungus (*N. coenophialum*; formerly *Acremonium coenophialum* Morgan-Jones and Gams) results in improved persistence in the seasonally drought- and heat-stressed region of the southeastern USA (Hill et al., 1991; Bouton et al., 1993). Reasons for greater persistence have been attributed to physiological responses that confer greater drought tolerance (Belesky et al., 1987a; West et al., 1993) and production of ergot alkaloids that may reduce forage intake by grazing animals, thereby reducing grazing pressure (Hoveland et al., 1983; Read and Camp, 1986; Hill et al., 1990). Toxic effects of endophyte-infected tall fescue on grazing animals have been well documented (Stuedemann and Hoveland, 1988; Bacon and Hill, 1997). Ecologically, the presence of *N. coenophialum* may be important in deterring plant-parasitic nematode populations (West et al., 1988) and various plant diseases (Latch, 1997).

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Another ecologically important characteristic of the tall fescue–endophyte association may be the potential for greater soil organic C sequestration. Soil organic C and N concentrations were significantly enhanced and potential soil microbial activity reduced in tall fescue stands with high compared with low frequency of endophyte infection (Franzluebbers et al., 1999). These data suggest that soil microbial activity may be sufficiently reduced in the presence of endophyte infection, as to eventually lead to greater accumulation of soil organic C and N. Alternatively, it is possible that plant production may be enhanced in the presence of the endophyte (Arachevaleta et al., 1989; Hill et al., 1990) or that changes in botanical composition of pastures with time might alter C inputs to soil. For example, tall fescue stands without endophyte association do not always persist as well as those with endophyte infection (Read and Camp, 1986; Bouton et al., 1993), which could result in encroachment by other plants leading to different quantity and quality of plant residues supplied to soil. Changes in botanical composition, such as tall fescue decline with bermudagrass (*Cynodon dactylon* L.) encroachment, could have either positive or negative consequences on soil C and N cycling (Wedin and Tilman, 1996).

The conditions under which endophyte infection of tall fescue might alter soil C and N cycling are currently limited to knowledge of endophyte effects on plant response variables (Malinowski and Belesky, 2000). Increased soil N availability may enhance accumulation of ergot alkaloids in tall fescue leaves (Lyons and Bacon, 1984; Belesky et al., 1988), and therefore could be expected to exacerbate differences in ergot alkaloid production between low- and high-endophyte-infected stands. In contrast, root and shoot dry matter production of endophyte-infected tall fescue plants responded less to increasing soil P availability than endophyte-free tall fescue plants (Malinowski et al., 1998). Therefore, the interaction of soil fertility with endophyte infection could be expected to have complex effects on soil C and N pools. In addition, the effects on soil C and N pools under pastures with varying levels of (i) endophyte infection frequency and (ii) ground cover due to natural pasture development are largely unknown.

Since soil microbial activity per unit of soil organic C was found to be inhibited by high compared with low endophyte infection (Franzluebbers et al., 1999), soil fractions rich in biologically active C and N pools could be expected to be altered most extensively. Water-stable macroaggregates (>0.25 mm) often contain higher quantities of mineralizable C and N than microaggregates (Elliott, 1986; Beare et al., 1994). Small macro-

Abbreviations: K-31, 'Kentucky-31'.

aggregates (0.25–1.0 mm) are sometimes more enriched in biologically active C than large macroaggregates (1.0–4.75 mm) (Franzluebbers and Arshad, 1997).

We hypothesized that endophyte infection would increase the stock of soil organic C and N and decrease the size and activity of soil microbial biomass C and N, but that long-term differences in soil fertility might alter these responses. Further, difference in pasture composition due to progressive tall fescue decline in low-endophyte-infected plantings was hypothesized to partially explain changes in soil organic C and N pools in mature (>10 yr) tall fescue pastures. The objective of this investigation was to quantify soil C and N pools under 20-yr-old tall fescue pastures varying in endophyte infection frequency, fertilization history, and cultivar.

MATERIALS AND METHODS

Site Characteristics

A field experiment was initiated in autumn 1981 near Watkinsville, GA (33°62′ N, 83°25′ W) on a Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludults). Long-term mean annual temperature for the area is 16.5°C, precipitation is 1250 mm, and pan evaporation is 1560 mm. During the period 1981 to 2001, yearly precipitation was 1220 ± 233 mm.

Experimental Setup and Management

The experimental design was a randomized complete block design with three replications of six treatments, as described earlier in Belesky et al. (1988). Treatments included (i) low-endophyte-infected K-31 tall fescue (<7% seed infection) with low fertilization (13.4–1.5–5.6 g N–P–K m^{-2} yr^{-1}), (ii) low-endophyte-infected K-31 tall fescue with high fertilization (33.6–3.7–13.9 g N–P–K m^{-2} yr^{-1}), (iii) high-endophyte-infected K-31 tall fescue (80% seed infection) with low fertilization, (iv) high-endophyte-infected K-31 tall fescue with high fertilization, (v) low-endophyte-infected AU-Triumph (Triumph) tall fescue with low fertilization, and (vi) low-endophyte-infected Johnstone tall fescue with low fertilization. Each of the 18 paddocks (0.7 ha) was grazed periodically with Angus cattle each year following establishment, primarily in spring and autumn, when growth of tall fescue was most vigorous. Paddock design was described in Wilkinson et al. (1989). All fertilization was suspended after 1997 to help avoid further accumulation of inorganic N in the soil profile below 0.3 m (Franzluebbers et al., 2000a). Termination of fertilizer application during the final 4 yr was not expected to greatly alter relative differences in soil organic C and N pools among treatments, which would have developed during the first 16 yr of this experiment.

Sampling and Analyses

Tall fescue tillers were collected yearly from 1983 to 1996 for determination of endophyte-infection frequency according to the procedure described in Belesky et al. (1987b). Six to 20 tillers per paddock were randomly collected during each of 7 ± 4 mo of the year. Although we did not measure alkaloid production throughout the course of the experiment, Belesky et al. (1988) showed that ergopeptine alkaloid levels in tall fescue leaf tissue were two- to threefold greater under high than low endophyte infection.

Basal ground cover (i.e., botanical composition of pasture) was evaluated in May 2001 from 30 areas (0.25 m^2 each) separated by >10 m in each paddock, all by the same experienced technician. Percentages of basal area (with separations in multiples of five) were calculated for the following six classes: (i) tall fescue, (ii) winter annual grass, (iii) bermudagrass, (iv) *Paspalum* spp., (v) broadleaves, and (vi) bare ground. None of the paddocks had measurable composition of *Paspalum* spp.

Soil was collected at depths of 0 to 3, 3 to 6, 6 to 12, and 12 to 20 cm in May 2002. Each paddock was divided into two zones for sampling: (i) a 0.5-ha area that included shade and water sources, and (ii) a 0.2-ha area farthest away from shade and water sources. Soil was composited from eight randomly located subsampling sites in Zone 1 and from five randomly located subsampling sites in Zone 2. Following removal of forage above 4 cm, surface residue from individual 0.04-m^2 subsampling sites was cut to the mineral surface with battery-powered hand shears, bagged, and dried at 55°C for several days. A 4-cm-diam. soil core was collected from each of the subsampling sites and sectioned into depth increments. Soil was oven-dried (55°C, 72 h), weighed, and gently crushed to pass a 4.75-mm screen to partially homogenize sample and remove stones (<1% of weight).

Soil bulk density was calculated from the oven-dried soil weight and pooled-core volume (1.88 to 8.04 × 10^{-4} m^3, depending on depth and zone). Soil for subsequent analyses was stored dried at ambient conditions. A subsample was ground in a ball mill to a fine powder and analyzed for total C and N with dry combustion (Leco CNS-2000, St. Joseph, MI).[1] It was assumed that total C was equivalent to organic C because soil pH was near 6.

Mineralizable C was determined from two subsamples of soil (27.5 g each for 0- to 3-cm depth, 48 g each for 3- to 6-cm depth, and 65 g each for 6- to 12- and 12- to 20-cm depths) wetted to 50% water-filled pore space (Franzluebbers, 1999), placed into a 1-L canning jar along with vials containing 10 mL of 1 *M* NaOH to trap evolved CO_2 and water to maintain humidity, and incubated at 25 ± 1°C for 24 d. Alkali traps were replaced at 3 and 10 d. Evolved CO_2 was calculated by titrating alkali with 1 *M* HCl to a phenolphthalein endpoint following precipitation of carbonate with excess $BaCl_2$. At 10 d of incubation, one of the subsamples was removed, fumigated for 24 h with $CHCl_3$, aerated, placed into a separate canning jar along with alkali and water, and incubated for 10 d at 25°C. Soil microbial biomass C was calculated from the quantity of CO_2 evolved during 10 d following fumigation divided by an efficiency factor of 0.41 (Voroney and Paul, 1984). Mineralizable N was determined from the same incubation as for C, representing the difference in inorganic N concentration between 0 and 24 d of incubation. Inorganic N (NH_4–N + NO_2–N + NO_3–N) was determined from the filtered extract of a 10-g subsample of oven-dried (55°C, 48 h) and sieved (<2 mm) soil shaken with 20 mL of 2 *M* KCl for 30 min using salicylate-nitroprusside and Cd-reduction autoanalyzer techniques (Bundy and Meisinger, 1994).

Particulate organic C and N were determined by shaking the oven-dried (55°C, 72 h) fumigated sample previously used for microbial biomass determination with 0.01 *M* $Na_4P_2O_7$ for 16 h, collecting the sand plus organic matter retained on a 0.053-mm screen, oven-drying (55°C, 72 h), weighing, grinding to a fine powder, and determining the C and N concentration using dry combustion as described previously.

For aggregate distribution and stability analyses, soil was initially oven-dried (55°C) and gently crushed to pass a 4.75-mm screen (Franzluebbers et al., 2000b). Dry aggregate

[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

distribution was determined by placing either a 50-g (0- to 3- and 3- to 6-cm depths) or a 100-g (6- to 12- and 12- to 20-cm depths) portion of soil on top of a nest of sieves (200-mm diam. with openings of 1.0, 0.25, and 0.053 mm), shaking for 1 min at Level 6 on a sieve shaker (CSC Scientific Company, Fairfax, VA, Catalogue No. 18480), and weighing soil retained on the screens and that passing the 0.053-mm screen. Water-stable aggregate distribution was determined from the reconstituted sample used for dry aggregate distribution placed on top of a nest of sieves (175-mm diam. with openings of 1.0 and 0.25 mm), immersed directly in water, and oscillated for 10 min (20-mm stroke length, 31 cycles min^{-1}). The two sieves were placed in an oven to dry (55°C, 24 h). Water and soil passing the 0.25-mm sieve was poured over a 0.053-mm sieve, with retained soil washed with a gentle stream of water and transferred into a drying bottle using a small stream of water. The <0.053-mm fraction was calculated as the difference between initial soil weight and summation of the other fractions. All fractions were oven-dried at 55°C for >24 h following visual dryness.

Mean-weight diameter of both dry- and water-stable aggregates was calculated by summing the products of aggregate fractions and mean diameter of aggregate classes. Macroaggregates were defined as soil retained on 1.0- and 0.25-mm sieves. Large macroaggregates were defined as soil retained on the 1.0-mm sieve. Stability of macroaggregates was calculated as the fraction of water-stable macroaggregates divided by the fraction of dry-stable macroaggregates. Stability of mean-weight diameter was calculated as water-stable mean-weight diameter divided by dry-stable mean-weight diameter.

Total C and N of the 1.0- to 4.75-, 0.25- to 1.0-, and 0.053- to 0.25-mm water-stable aggregate fractions were determined using dry combustion as described previously. The 1.0–4.75-mm fraction was ball milled to a fine powder before analysis, but other fractions were not.

Data from Zones 1 and 2 within a paddock were averaged and not considered a source of variation in the ANOVA using the general linear models procedure (SAS Institute, 1990). A priori orthogonal contrasts and unprotected least significant differences were used to separate means. Concentration of various soil properties (e.g., mg g^{-1}) was converted to content (g m^{-2}) using bulk density and soil volume. Analysis of variance for soil properties was conducted separately by cumulative-profile increments according to a randomized complete block design. Soil properties in cumulative-profile increments were from the sums of individual depth contents. Effects were considered significant at $P < 0.1$. Linear regression of endophyte-infection frequency on year of sampling was used to compute an average change. Correlations among soil and plant properties were determined using paddock means ($n = 18$) and declared significant only at $P < 0.01$.

RESULTS AND DISCUSSION

Pasture Characteristics

Endophyte-infection frequency of tall fescue tillers was significantly greater in high- than in low-endophyte-infection paddocks, as was expected from the initial experimental setup (Table 1). However, there was a steady increase ($P < 0.001$) in endophyte-infection frequency with time in all treatments, except under (i) K-31 with low endophyte infection–high fertilization and (ii) Triumph (Fig. 1). The yearly change in endophyte-infection frequency was 1.9 and 1.2% yr^{-1} (values different at $P < 0.1$) under K-31 with low and high endophyte infection–low fertilization, 0.6 and 2.3% yr^{-1} (values different at $P < 0.01$) under K-31 with low and high endophyte infection–high fertilization, and 0.1 and 2.3% yr^{-1} (values different at $P < 0.001$) under Triumph and Johnstone.

Ground cover in May 2001 was significantly affected by the six tall fescue treatments (Table 1). Basal area of tall fescue was greater under high than under low endophyte infection of K-31, averaging 74 and 54% across fertilization regimes, respectively. This result with long-term management supports results obtained in short-term studies (<3 yr), where tall fescue composition has been positively related to endophyte infection (Read and Camp, 1986; Bouton et al., 1993; Joost, 1995). Endophyte-infection frequency of tall fescue tillers at the end of 1996 was highly correlated to basal area of tall fescue in 2001 ($r = 0.82$, $P < 0.001$). Stand reduction of tall fescue with low endophyte infection of K-31 was primarily substituted with bermudagrass and winter annual grass. There was no difference in basal area of broadleaves and bare ground between low and high endophyte infection of K-31. With increased fertilization, basal area of tall fescue was reduced when averaged across endophyte infection levels, resulting in bermudagrass encroachment. Basal area of tall fescue was lowest in Triumph pastures (28%) possibly due to main-

Table 1. Endophyte-infection frequency and basal area of various botanical components of pastures in May 2001 as affected by cultivar, fertilization, and initial endophyte treatment.

			Endophyte infection frequency		Basal area				
Cultivar	Fertilization	Initial endophyte treatment level	Mean (1983–1996)	End of 1996	Tall fescue	Bermudagrass	Winter annuals	Broadleaves	Bare ground
			%						
K31	low	low	31	45	59	14	7	6	14
K31	low	high	65	75	82	3	<1	3	13
K31	high	low	25	29	50	28	8	5	10
K31	high	high	59	76	66	14	1	4	15
Triumph	low	low	1	2	28	22	6	24	20
Johnstone	low	low	26	43	45	23	9	9	15
LSD (0.1)			7	7	18	13	5	9	6
Source of variation		df	Pr > F						
K31 vs. others		1	<0.001	<0.001	0.001	0.10	0.07	0.003	0.07
Triumph vs. Johnstone		1	<0.001	<0.001	0.12	0.93	0.31	0.009	0.16
K31, low vs. high endophyte		1	<0.001	<0.001	0.02	0.03	0.006	0.48	0.49
K31, low vs. high fertilization		1	0.06	0.02	0.10	0.03	0.81	0.96	0.58
K31, endophyte × fertilization		1	0.90	0.008	0.62	0.85	0.93	0.74	0.23

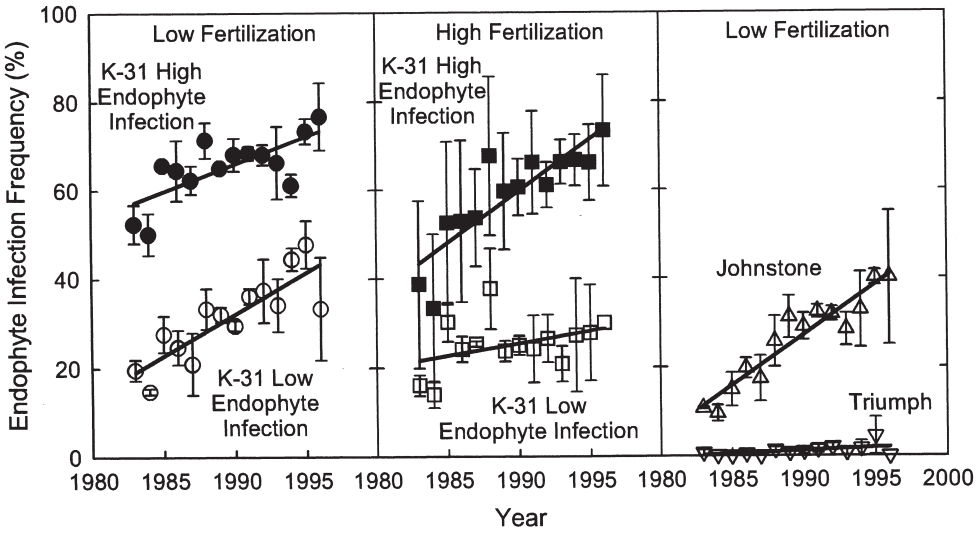

Fig. 1. Endophyte infection frequency of tall fescue tillers during the period of 1983 to 1996 as affected by endophyte infection level at planting (low = 7% seed infection, high = 80% seed infection), fertilization (low = 13.4–1.5–5.6 g N–P–K m^{-2} yr^{-1}, high = 33.6–3.7–13.9 g N–P–K m^{-2} yr^{-1}), and cultivar (Triumph and Johnstone). Lines represent linear regression, the results of which are specified in the text.

tenance of very low endophyte-infection frequency throughout the study. The loss of tall fescue stand with Triumph brought in nearly equal percentages of bermudagrass, broadleaves, and bare ground.

Whole-Soil Carbon and Nitrogen Pools

Endophyte Effect

Soil bulk density was not significantly different among pasture treatments at any soil depth (Table 2). However, soil bulk density did increase with depth in all pastures, as is typical for soils of this region (Franzluebbers et al., 2000b).

Significant effects of endophyte infection on whole-soil C and N pools occurred and were mostly due to comparisons under high fertilization. Pastures under high endophyte infection of K-31 had higher soil organic C than under low endophyte infection at a depth of 0 to 12 cm when averaged across fertilization regimes, but this effect was mostly due to the response under high fertilization (Table 2). A similar change occurred for total soil N at a depth of 0 to 20 cm (Table 3). Contents of all other organic C and N pools, including particulate, microbial biomass, and mineralizable, were not significantly affected by endophyte infection of K-31 under either fertilization regime (Tables 2–4). However, ratios of several biologically active C and N pools per unit of soil organic C and total N were significantly reduced under high than under low endophyte infection of K-31, especially with high fertilization (Table 5). Further, the ratio of CO_2 flush following rewetting of dried soil during 3 d of incubation/microbial biomass C (considered the active microbial biomass pool) was reduced under high than under low endophyte infection. The reduction in biologically active C and N pools with higher endophyte infection points to some inhibitory effect on soil microbial activity.

The influences of endophyte infection on soil C and N pools were mostly consistent with the effects reported previously from a study that included four paddocks not sampled here and eight of the 18 paddocks of this study, but sampled five years earlier (Franzluebbers et

Table 2. Soil bulk density, total organic C, and particulate organic C within cumulative-profile depths to 20 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Soil bulk density at depth, cm				Soil organic C at depth, cm				Particulate organic C at depth, cm			
			0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20
			Mg m^{-3}				g m^{-2}							
K31	low	low	1.17	1.31	1.42	1.49	1171	1915	2878	3724	534	825	1199	1405
K31	low	high	1.13	1.28	1.40	1.48	1173	1913	2968	3795	502	775	1158	1381
K31	high	low	1.17	1.30	1.43	1.48	1263	1987	2984	3872	651	939	1308	1534
K31	high	high	1.16	1.32	1.43	1.49	1364	2165	3277	4197	616	934	1315	1572
Triumph	low	low	1.13	1.29	1.42	1.47	1372	2169	3256	4108	659	965	1241	1421
Johnstone	low	low	1.35	1.39	1.46	1.51	1305	2012	3026	3915	553	822	1156	1382
LSD(0.1)			0.34	0.18	0.09	0.06	272	260	269	330	157	134	152	182
Source of variation		df	Pr > *F*											
K31 vs. others		1	0.50	0.51	0.57	0.68	0.32	0.30	0.24	0.33	0.58	0.59	0.39	0.27
Triumph vs. Johnstone		1	0.27	0.37	0.44	0.24	0.66	0.30	0.15	0.32	0.25	0.08	0.34	0.71
K31, low vs. high endophyte		1	0.88	0.95	0.79	0.96	0.64	0.41	0.10	0.16	0.59	0.62	0.78	0.93
K31, low vs. high fertilization		1	0.93	0.83	0.69	0.99	0.21	0.14	0.08	0.06	0.09	0.03	0.05	0.05
K31, endophyte × fertilization		1	0.89	0.77	0.83	0.76	0.65	0.39	0.36	0.35	0.98	0.68	0.69	0.67

Table 3. Total soil N, mineralizable N, and residual inorganic N within cumulative-profile depths to 20 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Total soil N at depth, cm				Mineralizable N at depth, cm				Residual inorganic N at depth, cm			
			0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20
			g m^{-2}				g m^{-2} 24 d^{-1}				g m^{-2}			
K31	low	low	78	127	191	225	4.1	6.3	8.4	10.8	0.7	1.3	2.2	3.3
K31	low	high	80	130	199	234	3.7	5.8	8.2	9.8	0.8	1.4	2.4	3.5
K31	high	low	87	139	203	242	4.9	6.9	9.2	10.4	0.8	1.5	2.5	3.8
K31	high	high	96	148	221	266	4.5	6.6	8.8	10.4	0.7	1.3	2.2	3.2
Triumph	low	low	93	149	222	260	4.2	6.0	8.2	10.4	0.9	1.5	2.5	3.6
Johnstone	low	low	91	143	207	248	3.7	5.8	8.5	10.0	0.9	1.6	2.7	4.0
LSD (0.1)			21	21	20	23	1.1	1.2	1.0	1.9	0.2	0.2	0.3	0.4
Source of variation		df	Pr > *F*											
K31 vs. others		1	0.35	0.19	0.14	0.16	0.30	0.22	0.50	0.84	0.11	0.04	0.02	0.03
Triumph vs. Johnstone		1	0.88	0.59	0.22	0.36	0.40	0.78	0.62	0.70	0.97	0.82	0.39	0.11
K31, low vs. high endophyte		1	0.48	0.43	0.12	0.09	0.39	0.42	0.47	0.49	0.72	0.56	0.69	0.29
K31, low vs. high fertilization		1	0.17	0.09	0.05	0.02	0.09	0.18	0.10	0.94	0.71	0.68	0.76	0.68
K31, endophyte × fertilization		1	0.70	0.72	0.56	0.43	0.99	0.78	0.79	0.54	0.63	0.23	0.09	0.06

al., 1999). These consistent effects were observed for soil organic C, total soil N, ratio of microbial biomass/soil organic C, ratio of mineralizable/total soil N, and ratio of CO_2 flush following rewetting/microbial biomass C. In the current study, however, we found that the endophyte effects were stronger in pastures with high fertilization. It appears that inhibitory effects on soil microbial activity and enhanced accumulation of soil organic C and total N with high endophyte infection may be exacerbated with higher soil fertility. The absolute difference in endophyte infection frequency between low and high endophyte levels of K-31 became greater with time under high than under low fertilization (Fig. 1). These data, combined with composition of pasture as tall fescue (Table 1), led to a greater relative proportion of endophyte-infected plants under high fertilization (3.3 times greater, in which 50% of pasture contained endophyte-infected plants under high endophyte infection level vs. 15% under low endophyte infection level) than under low fertilization (2.3 times greater, in which 62% of pasture contained endophyte-infected plants under high endophyte infection level vs. 27% under low endophyte infection level). Therefore, the impact of endophyte infection on soil C and N pools was positively associated with long-term exposure of soil to endophyte-infected plants, the quantity of which, was modified by soil fertility.

Particulate/total organic C and N were both reduced under high than under low endophyte infection of K-31 (Table 5). This observation partially contradicts previous findings of Franzluebbers et al. (1999). In this earlier study, particulate organic C and N were greater with high than with low endophyte infection, although the ratios of particulate/total organic C and total N were not significantly different. Particulate organic C and N below the residue-enriched surface soil can be considered an indicator of root proliferation, which would be expected to be greater under high than low endophyte infection (De Battista et al., 1990).

Fertilization Effect

The influence of long-term previous fertilization on soil C and N pools was limited. Increased fertilization resulted in significantly greater (8 ± 2%) soil organic C and total N at a depth of 0 to 12 and 0 to 20 cm (Tables 2 and 3). A similar fertilization effect was observed for particulate organic C, but not for particulate organic N. Residual inorganic N in surface soil was low in all pastures, but greater with increased fertilization under low endophyte infection (Table 3).

Table 4. Soil microbial biomass C, mineralizable C, and the flush of CO_2 during 3 d following rewetting within cumulative-profile depths to 20 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Soil microbial biomass C at depth, cm				Mineralizable C at depth, cm				Flush of CO_2-C at depth, cm			
			0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20
			g m^{-2}				g m^{-2} 24 d^{-1}				g m^{-2} 3 d^{-1}			
K31	low	low	60	91	129	158	64	96	128	152	22	32	44	53
K31	low	high	62	96	134	160	66	97	133	156	23	34	45	54
K31	high	low	65	96	135	161	65	96	130	154	22	33	44	53
K31	high	high	59	90	127	161	58	89	124	147	20	31	43	51
Triumph	low	low	63	93	133	160	60	89	121	144	21	31	42	51
Johnstone	low	low	59	89	127	159	71	99	132	155	24	34	46	54
LSD (0.1)			16	16	16	22	15	14	17	18	5	6	7	8
Source of variation		df	Pr > *F*											
K31 vs. others		1	0.94	0.69	0.83	0.95	0.65	0.98	0.74	0.70	0.69	0.97	0.96	0.99
Triumph vs. Johnstone		1	0.67	0.61	0.47	0.92	0.22	0.23	0.26	0.28	0.24	0.29	0.31	0.45
K31, low vs. high endophyte		1	0.75	0.92	0.81	0.97	0.66	0.71	0.93	0.78	0.83	0.93	0.94	0.97
K31, low vs. high fertilization		1	0.85	0.86	0.94	0.80	0.55	0.48	0.60	0.60	0.72	0.67	0.71	0.62
K31, endophyte × fertilization		1	0.52	0.39	0.30	0.91	0.44	0.51	0.43	0.45	0.45	0.48	0.51	0.58

Table 5. Ratios of various soil C and N pools at a depth of 0 to 12 cm as affected by endophyte level (LE is low endophyte and HE is high endophyte infection level) under two different fertilization regimes.

	Low fertilization			High fertilization			Mean		
Soil property	LE	Pr > *F*	HE	LE	Pr > *F*	HE	LE	Pr > *F*	HE
Particulate/total organic C, g g^{-1}	0.41	0.22	0.39	0.43	0.09	0.40	0.42	0.05	0.39
Microbial biomass/total organic C, mg g^{-1}	44	0.83	45	45	0.01	39	45	0.05	42
Mineralizable/total organic C, mg g^{-1} 24 d^{-1}	44	0.94	45	43	0.10	38	44	0.25	41
Mineralizable/total organic C, mg g^{-1} 3 d^{-1}	15	0.88	15	15	0.13	13	15	0.32	14
Microbial biomass/particulate organic C, mg g^{-1}	108	0.38	116	105	0.42	98	107	0.96	107
Mineralizable/particulate organic C, mg g^{-1} 24 d^{-1}	108	0.41	115	101	0.61	96	104	0.82	106
Mineralizable/particulate organic C, mg g^{-1} 3 d^{-1}	37	0.44	39	34	0.72	33	35	0.76	36
Mineralizable/microbial biomass C, g g^{-1} 24 d^{-1}	1.00	0.98	0.99	0.96	0.81	0.98	0.98	0.88	0.99
Mineralizable/microbial biomass C, g g^{-1} 3 d^{-1}	0.44	0.83	0.45	0.45	0.01	0.39	0.45	0.05	0.42
Particulate/total N, g g^{-1}	0.66	0.15	0.58	0.62	0.23	0.56	0.64	0.07	0.57
Mineralizable/total N, mg g^{-1} 24 d^{-1}	43	0.39	41	44	0.10	39	44	0.09	40
Mineralizable/particulate organic N, mg g^{-1} 24 d^{-1}	66	0.30	74	73	0.87	72	70	0.53	73
C/N of total organic matter, g g^{-1}	15.1	0.79	14.9	14.7	0.74	14.8	14.9	0.97	14.9
C/N of particulate organic matter, g g^{-1}	9.5	0.40	10.3	10.4	0.64	10.8	10.0	0.36	10.6
C/N of mineralizable organic matter, g g^{-1}	16.0	0.80	16.3	14.6	0.99	14.6	15.3	0.85	15.5
C/N of large macroaggregates, 1.0–4.75 mm, g g^{-1}	12.6	0.53	12.4	11.9	0.99	11.9	12.2	0.65	12.2
C/N of small macroaggregates, 0.25–1.0 mm, g g^{-1}	11.2	0.88	11.1	11.3	0.99	11.3	11.2	0.91	11.2
C/N of microaggregates, 0.053–0.25 mm, g g^{-1}	15.6	0.61	15.3	16.1	0.09	15.0	15.9	0.12	15.2

Soil organic C content at a depth of 0 to 12 cm was positively correlated with the area of bare ground (r = 0.60, P = 0.01). More bare ground may have been a result of at least two phenomena: (i) lush growth of tall fescue clumps at various times of the year due to high fertilization and high endophyte infection, which would have shaded neighboring areas and prevented a more complete distribution of plant bases, and (ii) greater number of dung pats that might have temporarily smothered plants in small areas. Both of these possibilities would have likely enhanced C input to soil. The only other whole-soil property that was significantly affected by pasture characteristics was the ratio of mineralizable/total N, which declined (r = −0.59, P = 0.01) with increasing area of broadleaves. Although residue quality of broadleaves might be different than that of various grasses, and therefore affect mineralizable N, the occurrence and persistence of broadleaves in pastures are not likely stable with time. Various other biotic and abiotic factors would also likely play roles in forming this association.

Cultivar Effect

Although both Triumph and Johnstone were planted with similarly low endophyte infection frequency, tall fescue tillers in Johnstone pastures increased in endophyte infection frequency with time while tillers in Triumph pastures remained low (Fig. 1). Using these two cultivars as a surrogate for differences in endophyte infection frequency, soil C and N pools did not respond the same as compared with K-31 endophyte infection treatments (Tables 2–4). Particulate organic C at a depth of 0 to 6 cm was the only soil property that was significantly different between Triumph (2% infection) and Johnstone (43% infection). Besides differences in endophyte infection level, these two cultivars may have also differed in quantity, quality, timing, and architecture of various root and shoot properties that could have affected soil C and N properties.

The largest differences in soil organic C and total N among cultivars were between K-31 and Triumph, with soil under Triumph having 10 to 17% higher values. Because of the low basal area of tall fescue (28%) and low endophyte infection frequency (2%) in Triumph pastures, changes in soil C and N appear unlikely to have originated from a direct endophyte effect, but rather indirectly from changes in ground cover with time and forage quality. We did not measure forage quality among tall fescue cultivars before termination of the experiment in May 2002, but Hill et al. (1990) noted significant differences in forage quality among tall fescue genotypes. We observed a greater area of broadleaves and bare ground in Triumph pastures than in K-31 or Johnstone pastures (Table 1). The reason why Triumph pastures contained greater soil organic C and total N than the other two cultivars is not easily explained. If a greater percentage of bare area would have been derived from dung pats (not measured) that smothered forage, then there should have also been higher particulate organic C and N and residual inorganic N (Table 3). However, this was not observed.

Stability and Carbon and Nitrogen Content of Aggregates

Stability of macroaggregates (>0.25 mm) and stability of mean-weight diameter in water were both high in all pastures sampled (Table 6). Only at a depth of 12 to 20 cm did soil aggregates indicate any breakdown in water. These high aggregate stability indices under long-term tall fescue pastures are consistent with values reported for other conservation management systems in the same region (Franzluebbers et al., 2000b). Perhaps as a result of these overall high values, there were no differences in aggregate stability indices due to endophyte infection or fertilization.

Carbon and N contents of small macroaggregates (0.25–1.0 mm) were greater under high than under low endophyte infection of K-31 at a depth of 0 to 12 cm, but only with increased fertilization (Tables 7 and 8). These endophyte effects were mostly consistent with the changes that occurred in whole-soil total C and N. It is not clear as to how or why these changes in C and N due to endophyte infection under high fertility

Table 6. Soil aggregate distribution and stability within cumulative-profile depths to 12 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Water-stable macroaggregates (>0.25 mm) at depth, cm			Stability of macroaggregates at depth, cm			Mean-weight diameter of water-stable aggregates at depth, cm			Stability of mean-weight diameter at depth, cm		
			0–3	0–6	0–12	0–3	0–6	0–12	0–3	0–6	0–12	0–3	0–6	0–12
			g g^{-1}			g (wet) g (dry)$^{-1}$			mm			mm (wet) mm (dry)$^{-1}$		
K31	low	low	0.73	0.78	0.79	1.01	1.00	0.99	1.18	1.29	1.36	1.07	1.07	1.02
K31	low	high	0.76	0.78	0.79	1.00	0.99	0.99	1.16	1.29	1.35	1.06	1.04	1.02
K31	high	low	0.76	0.78	0.79	1.00	0.99	0.98	1.20	1.30	1.37	1.05	1.03	1.00
K31	high	high	0.75	0.78	0.79	1.01	1.00	1.00	1.10	1.27	1.31	1.07	1.06	1.05
Triumph	low	low	0.75	0.77	0.78	0.99	0.95	0.95	1.32	1.32	1.39	1.04	0.97	0.95
Johnstone	low	low	0.78	0.79	0.80	0.99	0.99	0.98	1.19	1.34	1.43	1.04	1.04	1.02
LSD(0.1)			0.03	0.04	0.04	0.03	0.04	0.03	0.16	0.20	0.24	0.07	0.07	0.06
Source of variation		df	Pr > F											
K31 vs. others		1	0.11	0.92	0.98	0.15	0.11	0.05	0.13	0.52	0.43	0.37	0.09	0.08
Triumph vs. Johnstone		1	0.13	0.30	0.27	0.76	0.10	0.12	0.18	0.81	0.75	0.83	0.12	0.08
K31, low vs. high endophyte		1	0.36	0.82	0.91	0.80	0.84	0.60	0.37	0.88	0.71	0.78	0.98	0.35
K31, low vs. high fertilization		1	0.38	0.95	0.97	0.82	0.86	0.85	0.79	0.97	0.86	0.87	0.69	0.94
K31, endophyte × fertilization		1	0.14	0.85	0.86	0.65	0.62	0.43	0.57	0.86	0.76	0.69	0.35	0.26

became more expressed within the small macroaggregate fraction.

Fertilization effects on soil C and N contents in small macroaggregates were significant only under high endophyte infection of K-31 (Table 7). With significant changes in C and N due to fertilization and endophyte infection only in small macroaggregates, this aggregate fraction should be considered a key location for further research in identifying pasture management effects on potential soil C sequestration, at least for Ultisols in the USA.

Cultivar effects on soil C and N in aggregate fractions were also significant only for small macroaggregates (Table 7). Carbon and N contents of small macroaggregates were greater under K-31 than under Triumph and Johnstone pastures.

The only pasture characteristic associated with soil aggregate properties was endophyte infection frequency at the end of 1996, which was positively correlated (r = 0.70, P = 0.001) with C and N contents in small macroaggregates at a depth of 0 to 12 cm. The C and N contents in small macroaggregates were the only soil properties that were consistently related to endophyte infection frequency, irrespective of fertilization and cultivar differences.

CONCLUSIONS

Tall fescue pastures had levels of endophyte infection ranging from 1 to 79% and had naturalized during 20 yr of management into mixed stands of tall fescue (28–82% of basal area), bermudagrass (3–28%), winter annual grass (1–9%), and broadleaves (3–24%). Soil organic C and total N were greater with high than with low endophyte infection of K-31 tall fescue and greater under high fertility. Biologically active organic matter (i.e., soil microbial biomass and mineralizable C and N) per unit of soil organic C and total N were depressed under high compared with low endophyte infection of K-31, suggesting some inhibition of the active pools of organic C and N by endophyte-infected tall fescue. The effect of endophyte infection on soil C and N pools was stronger under high fertility. Carbon and N contents of small macroaggregates (0.25–1.0 mm) were the most consistent and sensitive organic matter pool to endophyte infection, irrespective of fertilization regime or cultivar of tall fescue pastures. Accumulation of C and N due to endophyte infection occurred preferentially in macroaggregates, suggesting a biophysical mechanism of protection that should be explored further.

Table 7. Total organic C content in water-stable aggregate fractions within cumulative-profile depths to 20 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Large macroaggregates (1.0–4.75 mm) at depth, cm				Small macroaggregates (0.25–1.0 mm) at depth, cm				Microaggregates (0.053–0.25 mm) at depth, cm			
			0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20
			g m^{-2}											
K31	low	low	526	843	2047	2686	200	636	977	1471	82	114	177	342
K31	low	high	526	894	2216	2957	210	639	1000	1483	84	115	173	332
K31	high	low	565	925	2250	3045	190	591	940	1474	76	110	170	329
K31	high	high	516	884	2401	3041	212	702	1103	1669	100	135	194	358
Triumph	low	low	552	943	2454	3219	213	589	897	1348	70	110	166	345
Johnstone	low	low	616	1037	2310	2952	243	612	930	1399	89	120	179	342
LSD(0.1)			144	251	641	798	66	53	74	112	27	32	35	35
Source of variation		df	Pr > F											
K31 vs. others		1	0.33	0.25	0.49	0.58	0.29	0.04	0.005	0.003	0.51	0.75	0.64	0.80
Triumph vs. Johnstone		1	0.44	0.52	0.69	0.56	0.43	0.45	0.44	0.42	0.23	0.59	0.51	0.91
K31, Low vs. high endophyte		1	0.67	0.96	0.54	0.68	0.55	0.02	0.01	0.04	0.24	0.32	0.47	0.51
K31, Low vs. high fertilization		1	0.80	0.72	0.46	0.49	0.87	0.67	0.28	0.06	0.64	0.53	0.61	0.65
K31, endophyte × fertilization		1	0.68	0.65	0.97	0.67	0.82	0.03	0.04	0.06	0.31	0.37	0.31	0.20

Table 8. Total N content in water-stable aggregate fractions within cumulative-profile depths to 20 cm as affected by cultivar, fertilization, and initial endophyte treatment.

Cultivar	Fertilization	Initial endophyte treatment level	Large macroaggregates (1.0–4.75 mm) at depth, cm				Small macroaggregates (0.25–1.0 mm) at depth, cm				Microaggregates (0.053–0.25 mm) at depth, cm			
			0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20	0–3	0–6	0–12	0–20
			g m^{-2}											
K31	low	low	35	60	162	210	19	68	88	125	7	8	11	21
K31	low	high	34	64	179	229	20	67	90	124	7	8	11	21
K31	high	low	39	69	189	249	17	63	84	125	6	7	11	21
K31	high	high	36	66	203	252	20	75	98	139	8	10	13	23
Triumph	low	low	38	70	204	260	21	62	81	114	6	8	11	22
Johnstone	low	low	41	75	186	236	24	65	85	120	7	9	12	22
LSD(0.1)			11	21	55	71	7	7	7	13	2	3	3	3
Source of variation		df	Pr > *F*											
K31 vs. others		1	0.35	0.30	0.54	0.60	0.21	0.07	0.008	0.03	0.56	0.88	0.84	0.70
Triumph vs. Johnstone		1	0.69	0.68	0.57	0.55	0.47	0.38	0.30	0.46	0.22	0.48	0.41	0.71
K31, low vs. high endophyte		1	0.64	0.92	0.49	0.71	0.59	0.04	0.01	0.21	0.18	0.25	0.34	0.47
K31, low vs. high fertilization		1	0.51	0.48	0.25	0.29	0.82	0.53	0.50	0.16	0.78	0.66	0.72	0.61
K31, endophyte × fertilization		1	0.80	0.68	0.95	0.77	0.88	0.03	0.03	0.20	0.20	0.32	0.30	0.38

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