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Soil Carbon and Nitrogen Pools under Low- and High-Endophyte-Infected Tall Fescue

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ABSTRACT

Tall fescue (*Festuca arundinacea* Schreb.) is an important cool-season perennial forage for cattle production in the humid regions of the USA and throughout the world. While endophyte (*Neotyphodium coenophialum* Glenn, Bacon, & Hanlin) infection of tall fescue has many benefits, it also results in accumulation of toxic alkaloids in leaf tissue known to cause animal health disorders when ingested. We hypothesized that pastures containing these alkaloids may alter soil organic matter dynamics. A set of three grazed field experiments representing low (0–29%) and high (65–94%) endophyte infection of tall fescue was evaluated at the end of either 8 or 15 yr. Soil samples from 12 paddocks (0.7–0.8 ha) were collected at depths of 0 to 25, 25 to 75, 75 to 150, and 150 to 300 mm. Soil under tall fescue with high endophyte infection had 13 ± 8% greater concentrations of soil organic C and N and particulate organic N to a depth of 150 mm than with low endophyte infection. However, with high endophyte infection, microbial biomass and basal soil respiration per unit of soil organic C or particulate organic C were 86 ± 5% of those with low endophyte infection. Only small differences in soil microbial community structure, estimated via fatty acid methyl ester profiles, were observed between soils under fescue with 0 and 100% endophyte infection. Endophyte infection of tall fescue may perform an important ecological function, allowing more soil organic C and N to accumulate, perhaps because of reduced soil microbial activity on plant residues containing endophyte byproducts.

Tall fescue is an important cool-season perennial forage for many cattle producers in the humid regions of the USA and throughout the world. It is grown on more than 140 000 km^2 of land in the USA (Buckner et al., 1979). The majority of tall fescue pastures in the USA are infected with a fungus, *Neotyphodium coenophialum* (Shelby and Dalrymple, 1987), which resides primarily within basal stem tissue.

The *Neotyphodium*–tall fescue association is one of mutualism. Tall fescue provides *Neotyphodium* with energy, nutrients, shelter, and a means of propagation through the seed, while *Neotyphodium* provides mechanisms for improving tall fescue persistence by offering several biochemical deterrents to overgrazing and insect pressure (Bacon and Hill, 1997). Perhaps because of this association, endophyte-infected tall fescue persists with grazing better than other cool-season species in the southeastern USA. Cattle grazing endophyte-infected tall fescue can suffer from a number of health disorders, including fescue foot, fat necrosis, and fescue toxicosis (Stuedemann and Hoveland, 1988). Cattle grazing or fed tall fescue hay with a high endophyte level ingested 65 to 92% as much forage, produced 57 to 83% as much milk, gained 21 to 78% as much weight per day, and gained 65 to 89% as much weight per hectare compared with animals fed tall fescue having a low endophyte level (Stuedemann and Hoveland, 1988). Reduced animal production and performance is linked to toxic ergopeptine alkaloids, which accumulate in endophyte-infected tall fescue herbage (Stuedemann and Thompson, 1993). In addition to the negative effects on grazing cattle, endophyte infection may also deter insect herbivory. Although tall fescue has relatively few insect pest problems, endophyte-free perennial ryegrass (*Lolium perenne* L.), which also normally benefits from an endophytic association in nature, is rapidly consumed by herbivorous insects because of the absence of alkaloids, which would normally deter such activity (Prestidge et al., 1982; Latch, 1993; Rowan and Latch, 1994).

In addition to biochemical deterrents, *N. coenophialum* confers greater drought tolerance that could improve tall fescue productivity (Bouton et al., 1993; West

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et al., 1993). Suggested mechanisms for drought tolerance in endophyte-infected tall fescue are a lower net photosynthetic rate, higher stomatal resistance (Belesky et al., 1987a), and greater root proliferation under drought-stressed conditions (Richardson et al., 1990).

Toxic alkaloids in endophyte-infected tall fescue have deterrent effects on foraging cattle, herbivorous insects, pathogenic fungi, viruses, and root-feeding nematodes (Latch, 1997). Therefore, we hypothesized that these same alkaloids deposited to soil via plant litter, dung, and urine could alter microbial community structure and soil organic matter dynamics. We also hypothesized that the time under tall fescue and the fertilizer application level could alter the soil organic matter response to endophyte infection. Our objectives were to characterize the size of soil organic C and N pools (total, particulate, and microbial), potential microbial activity, and microbial community structure under tall fescue with low and high endophyte levels.

MATERIALS AND METHODS

Twelve tall fescue paddocks, separated into paired low and high endophyte levels, were established near Watkinsville, GA (33°62′ N lat; 83°25′ W long) on Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). The mean annual temperature is 16.5°C, precipitation is 1250 mm, and pan evaporation is 1560 mm. Duplicate paddocks were established for each of three sets of contrasting endophyte levels. One set of paddocks (0.7 ha each) was planted to (i) high-endophyte-infected Kentucky–31 tall fescue (80% seed infection) and (ii) low-endophyte-infected Kentucky–31 tall fescue (<7% seed infection) in autumn 1981 and reseeded again in spring 1982 to increase stand. Set 1 received annual applications of 33.6–3.7–13.9 as N–P–K (g m^{-2}). Another set of paddocks (Set 2) was established at the same time in the same way, except with annual applications of 13.4–1.5–5.6 as N–P–K (g m^{-2}). The third set of paddocks (0.8 ha each) was fertilized the same as the first set, but planted to 100%-infected tall fescue in autumn 1987 and to 0%-infected tall fescue in autumn 1988 (the same 100%-infected seed source was incubated at ambient temperature for 1 yr to reduce endophyte viability). All paddocks were grazed with Angus cattle each year following establishment, primarily in spring and autumn. For Sets 1 and 2, endophyte-infection frequency of tall fescue tillers, determined according to the procedure described in Belesky et al. (1987b), was not different between fertility levels, but increased with time under both endophyte infection levels (Fig. 1). For Set 3, endophyte-infection frequency of tillers did not change with time, averaging 0 and 94% from 1989 to 1996. 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 2- to 3-fold greater under high than under low endophyte infection early in this experiment.

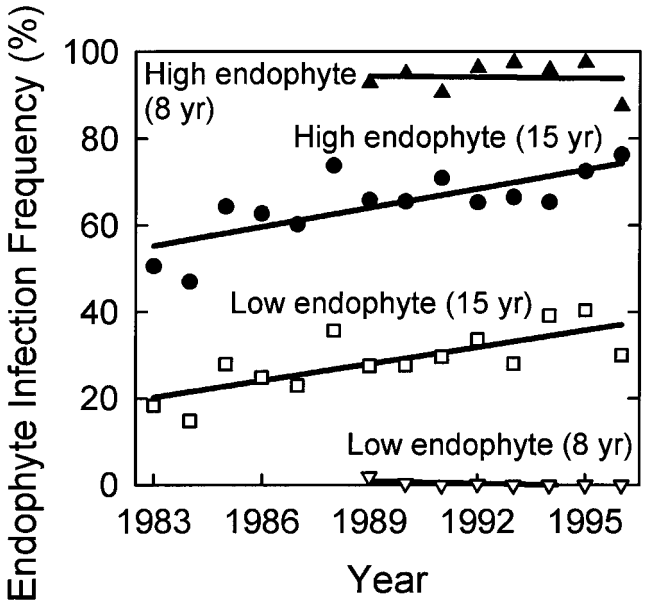

Fig. 1. Endophyte-infection frequency of tall fescue tillers with time.

Soil samples were collected at depths of 0 to 25, 25 to 75, 75 to 150, and 150 to 300 mm at distances of 1, 10, 30, 50, and 80 m from permanent shade and water sources, which were located 20 m apart along one edge of each paddock. Paddock design was described in Wilkinson et al. (1989). Paddocks in Set 3 were sampled 23 to 26 Jan. 1997. Paddocks in Sets 1 and 2 were sampled 11 to 13 Feb. 1997. During this 3-wk sampling period, rainfall totaled 54 mm and the mean daily air temperature was 8°C ± 4°C. During the preceding 3 wk in January, 103 mm of rainfall was received and the mean daily air temperature was 5°C ± 7°C. Tall fescue was green at the time of sampling, but growth was minimal. Eight cores (41-mm diam.) were composited within each depth and distance. Soil was oven-dried (55°C, 48 h), weighed, and crushed to pass a screen (4.75-mm openings) to partially homogenize the sample and remove stones (<1% of weight). Bulk density was calculated from the oven-dried soil weight and coring device volume. Soil for subsequent analyses was stored dried at ambient conditions. A subsample was ground 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.

Two subsamples of soil (15 g each for 0- to 25–mm depth, 40 g each for 25- to 75-mm depth, and 60 g each for 75- to 150-mm and 150- to 300-mm depths) were wetted to 50% water-filled pore space (Franzluebbers, 1999a), 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°C ± 1°C for 24 d to determine C mineralization (Franzluebbers and Arshad, 1996). 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. Basal soil respiration was calculated as the linear rate of respiration from 10 to 24 d of incubation and represented an estimate of potential microbial activity. 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). Determination of soil microbial biomass C following rewetting of dried soil with 10 d of pre-incubation has been shown to yield equivalent estimates compared with those from field-moist soil (Franzluebbers et al., 1996; Franzluebbers, 1999b).

Net N mineralization was determined from 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

[1] Trade and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the U.S. Department of Agriculture.

(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 0.06-mm screen, oven-drying (55°C, 72 h), weighing, grinding to a fine powder, and determining the C and N concentrations using dry combustion as described previously.

Soil microbial community structure was estimated from whole-soil fatty acid methyl esters (Cavigelli et al., 1995) at all four depths, but only in Set 3 at 30, 50, and 80 m (Replication 1) and 30 and 50 m (Replication 2) from shade and water. A standard protocol for determining whole-soil fatty acid methyl esters was not available at the time this research was undertaken. A protocol was developed based on coauthor experiences (Nazih, Hartel, and Fuhrmann, unpublished data, 1997), which was similar to that described in Cavigelli et al. (1995) and is described as follows. Drying of soil was rapid and was expected to limit fatty acid transformations during the 5-mo storage until extraction and analysis. Fatty acids were extracted from 3 g of dried soil following suspension in 15 mL of methanol–2 *M* KOH, incubation in a water bath at 37°C for 1 h with vortexing every 10 min for 20 s, neutralization of solution with 1 *M* acetic acid, addition of 10 mL of hexane with vortexing for 30 s, centrifugation at 480 × *g* for 20 min, collection of 6.6 mL of the hexane layer in a test tube, and evaporation in a water bath at 40°C with a gentle stream of N_2 gas to complete dryness. The extract was resuspended in 0.5 mL of 1:1 mixture (vol/vol) hexane and tert-butyl methyl ether (2-methoxy-2-methylpropane) and a 2-L portion was analyzed with an HP 5890 gas-liquid chromatograph (Hewlett Packard, Rolling Meadows, IL) equipped with an HP Ultra 2 capillary column (5%-diphenyl-95%-dimethylpolysiloxane, 25 m by 0.2 mm) and a flame ionization detector.

Whole-soil fatty acid methyl ester profiles were identified with the EUKARY program and naming table of MIDI (Microbial ID, Newark, DE). Concentrations of individual fatty acid methyl esters were subjected to analysis of variance using the general linear model procedure of SAS (SAS Institute, 1990). Although concentrations of fatty acid methyl esters were not absolute, semi-quantitative comparisons among soil depths and treatments are valid because a standard protocol was employed for all samples.

Fatty acid data were also examined by principal components analysis using CANOCO (Windows 4.0, Microcomputer Power, Ithaca NY) for each depth separately. Only named peaks using MIDI were expressed as a percentage of total named peak area from fatty acid methyl esters with ≤20 C chain lengths. Bacterial fatty acids are primarily <21 C chain lengths (Kennedy, 1994). Eukaryotic organisms also contain fatty acids in this range, but fatty acids >20 C chain lengths are more characteristic of eukaryotic than prokaryotic organisms (Cavigelli et al., 1995; White et al., 1997). Therefore, by focusing on fatty acid methyl esters ≤20 C chain lengths, we may have slighted eukaryotic microflora, but possibly reduced interference from plant and animal sources. We used a correlation matrix that gave all fatty acids equal weight in the principal components analysis, as opposed to a covariance matrix in which fatty acids were weighted proportional to their variance (Jongman et al., 1995). Our rationale was to prevent the more common, and hence often less useful, fatty acids from dominating the analysis and thereby masking more subtle shifts in the soil microbial fatty acid composition.

Analysis of variance was used to test the significance of difference in soil C and N pools between endophyte treatments at (i) each depth and (ii) across depths as a standing stock weighted by volume and bulk density using SAS (SAS Institute, 1990). Values were blocked according to replicate paddock and distance from shade and water sources within each set. Within-paddock sampling distances were included as blocked, experimental units to increase precision by providing more sampling units. Of the 23 soil properties (10 measured and 13 calculated ratios) at each of the four depths, 65% had lower between-paddock variability than within-paddock variability. The ratio of between-paddock/within-paddock variability was 0.90 ± 0.64, suggesting within-paddock sampling distances provided estimates that could be considered independent. Differences were considered significant at $P \leq 0.1$.

Results are presented as main effects of relative endophyte infection level averaged across experimental sets (i.e., fertilizer application level [13.4 or 33.6 g N m^{-2} under tall fescue for 15 yr] and time under tall fescue [8 or 15 yr]), as most interactions between endophyte level and experimental set were not significant. Integration of soil properties across depths was calculated to 150 mm only, rather than 300 mm, because most effects were significant within the 0- to 150-mm depth, but became less significant or not significant by dilution with soil below 150 mm.

RESULTS AND DISCUSSION

Soil Bulk Density

Soil bulk density was greater under tall fescue with low endophyte infection compared with high endophyte infection at a depth of 0 to 25 mm (Fig. 2). No differences in soil bulk density between endophyte levels occurred at other depths. Taken to a depth of 0 to 150 mm, soil bulk density was statistically greater under low than under high endophyte infection (Table 1), but this difference was probably of little practical significance. Soil organic C concentration under tall fescue with low endophyte infection (49 mg g^{-1} soil) was significantly lower ($P = 0.01$) than with high endophyte infection (58 mg g^{-1} soil) at a depth of 0 to 25 mm. Greater bulk density at the soil surface with low endophyte infection compared with high endophyte infection may have been due to lower soil organic C concentration resulting in compaction at the soil surface with animal traffic. Combination of greater bulk density and lower soil organic C concentration with low endophyte infection may have been a

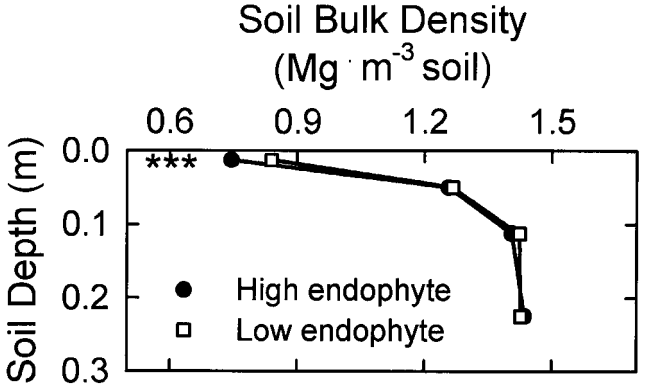

Fig. 2. Soil bulk density with depth under tall fescue as affected by endophyte infection level; (* indicates significance at $P \leq 0.001$).**

Table 1. Soil physical, chemical, and biological properties to a depth of 150 mm under tall fescue pasture as affected by *Neotyphodium coenophialum* infection.

Soil property	Endophyte infection level		
	High		Low
Bulk density (Mg m^{-3})	1.25	*	1.27
C mineralization (g CO_2–C m^{-2})$_{0\text{-}3\ d}$	47.6	*	53.6
C mineralization (g CO_2–C m^{-2})$_{0\text{-}24\ d}$	144	*	164
Basal soil respiration (BSR, g C m^{-2} d^{-1})	4.85	‡	5.39
Net N mineralization (NMIN, g m^{-2})$_{0\text{-}24\ d}$	6.86	ns	6.43
Soil microbial biomass C (SMBC, g m^{-2})	191	ns	192
Particulate organic C (POC, kg m^{-2})	1.24	*	1.11
Particulate organic N (PON, g m^{-2})	59.8	**	49.4
Soil organic C (SOC, kg m^{-2})	3.12	**	2.91
Total N (TN, g m^{-2})	229	**	211
Mineralizable C-to-N (g g^{-1})$_{0\text{-}24\ d}$	23.0	*	30.5
BSR-to-SMBC (mg g^{-1} d^{-1})	25.9	*	28.7
BSR-to-POC (mg g^{-1} d^{-1})	3.91	***	4.89
BSR-to-SOC (mg g^{-1} d^{-1})	1.56	**	1.87
NMIN-to-SMBC (mg g^{-1} 24 d^{-1})	36.3	ns	34.6
NMIN-to-PON (mg g^{-1} 24 d^{-1})	119	†	135
NMIN-to-TN (mg g^{-1} 24 d^{-1})	29.6	ns	30.1
SMBC-to-POC (mg g^{-1})	154	**	172
SMBC-to-SOC (mg g^{-1})	60.6	ns	66.2
POC-to-SOC (mg g^{-1})	398	ns	388
PON-to-TN (mg g^{-1})	259	ns	238

ns, †, *, **, and *** denote not significant and significant at $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

result of less undigested plant litter entering the soil because forage intake may have been greater than with high endophyte infection. However, differences in grazing pressure between endophyte treatments should have been minimal in Sets 1 and 2, which were grazed by a put-and-take system to maintain similar forage availability. When differences in cattle density occurred, the put-and-take system allowed more cattle on paddocks with high endophyte level (data not shown). Set 3 was managed by standard stocking density without overgrazing, which could have led to differences in grazing pressure between treatments.

Total and Particulate Organic Carbon and Nitrogen

Soil organic C and total N on a volumetric basis were not different between endophyte levels at a depth of 0 to 25 mm, but were lower under tall fescue with low endophyte infection compared with high endophyte infection at a depth of 25 to 75 mm (Fig. 3). To a depth of 150 mm, soil organic C was 6 ± 5% lower (mean ± SD among three experimental sets) and total N was 8 ± 2% lower with low endophyte infection compared with high endophyte infection (Table 1).

Particulate organic N on a volumetric basis was lower under tall fescue with low endophyte infection compared with high endophyte infection at depths of 0 to 25 and 25 to 75 mm (Fig. 4). Particulate organic C was lower with low endophyte infection compared with high endophyte infection at depths of 25 to 75 mm and 75 to 150 mm (Fig. 4). Although the ratio of particulate organic C/soil organic C was unaffected by endophyte level at all soil depths, the ratio of particulate organic N/total N was lower with low endophyte infection compared with high endophyte infection at depths of 0 to 25 and 25 to 75 mm (data not shown). Similar to that observed for soil organic C and total N to a depth of 150 mm, particulate organic C and N were lower with low endophyte infection compared with high endophyte infection (Table 1). Particulate organic N was more sensitive to the presence of endophyte infection than either particulate organic C or total N within each depth increment and to a depth of 150 mm. Less particulate organic C and N under tall fescue with low endophyte infection compared with high endophyte infection could indicate less root proliferation of tall fescue, as this fraction may express the contribution of roots more directly than does soil organic C and total N. Less vigorous rooting under endophyte-free than under endophyte-infected tall fescue has been suggested previously (Richardson et al., 1990). In a rhizotron study, root length was reduced under endophyte-free tall fescue to a depth of 1 m compared with endophyte-infected tall fescue during 1 of 2 yr (Knox, 1994). It appears that particulate organic C and N could be potential indicators of rooting, especially in soil below the surface residue layer (e.g., below 25 mm). Further work is needed to develop these quantitative relationships.

Soil Microbial Biomass Carbon

Endophyte infection had only a minimal effect on soil microbial biomass C when averaged across experimental sets differentiated by fertilizer application and time of establishment. Soil microbial biomass C was the only soil property to exhibit an interaction between endophyte level and experimental set (Fig. 5). At a depth of 25 to 75 mm, soil microbial biomass C was (i) 17% lower ($P = 0.03$) with low endophyte infection compared with

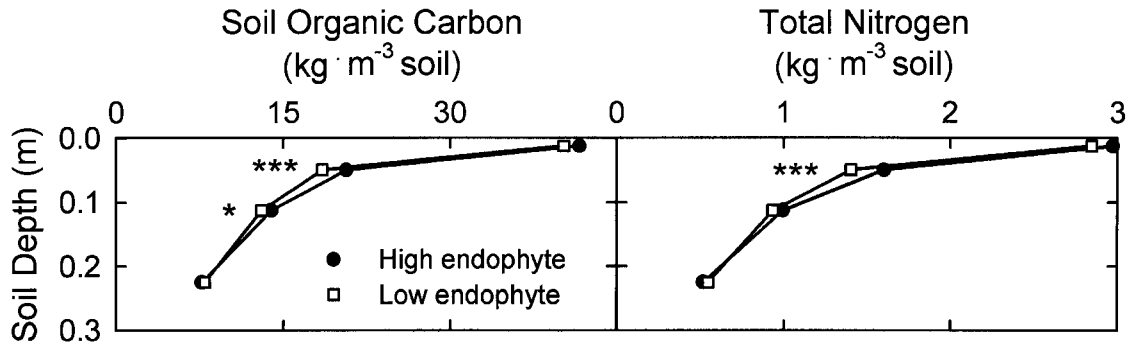

Fig. 3. Soil organic C and total N with depth under tall fescue as affected by endophyte infection level; (* and * indicate significance at $P \leq 0.05$ and $P \leq 0.001$, respectively).**

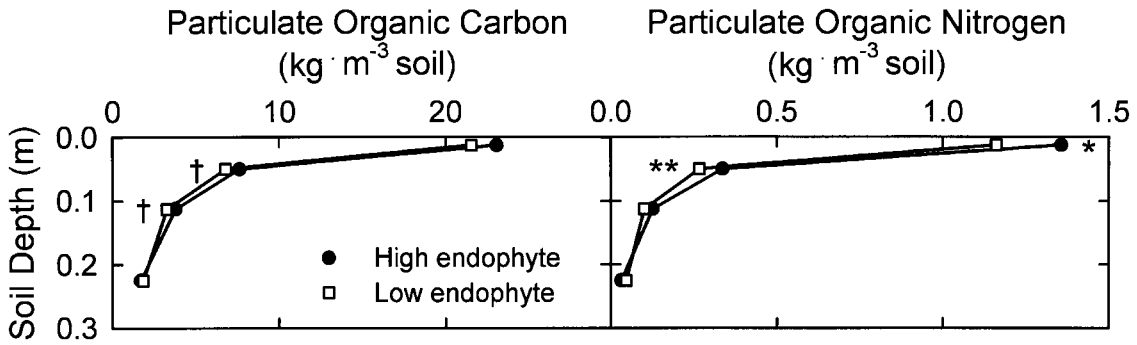

Fig. 4. Particulate organic C and N with depth under tall fescue as affected by endophyte infection level; (†, *, and ** indicate significance at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively).

high endophyte infection under tall fescue grown for 15 yr with high fertility (ii) 7% lower ($P = 0.40$) with low endophyte infection compared with high endophyte infection at the end of 15 yr with low fertility, and (iii) 24% greater ($P = 0.16$) under endophyte-free tall fescue than under endophyte-infected tall fescue at the end of 8 yr with high fertility. It is unclear why microbial biomass reacted differently to endophyte infection among experimental sets at this depth. The observed interaction may have been due to the length of time in tall fescue or due to the intensity of infection level. Perhaps soil microbial biomass adapts gradually to accumulation of endophyte-infected tall fescue residues, such that microbial biomass is suppressed initially by various inhibiting compounds, but enhanced later when microbial populations adapt to stockpiled substrates. Also the strong contrast between 0 and 100% infection may have been necessary to express any negative impacts of endophyte infection on microbial biomass. Further research is needed to better understand this interaction.

Potential Microbial Activity

Basal soil respiration was greater under tall fescue with low endophyte infection compared with high endophyte infection at a depth of 0 to 25 mm only (Fig. 6). This same pattern was observed with cumulative C mineralization during 24 d (data not shown). In general, basal soil respiration is usually tightly linked to soil microbial biomass and organic C (Franzluebbers et al., 1994, 1999a). Our observation of greater basal soil respiration, but lower soil organic C with low endophyte infection compared with high endophyte infection supports a conclusion that high endophyte infection had decreased the quality of organic substrate, allowing more soil organic C to accumulate. Endophyte infection of tall fescue may have inhibited microbial activity, at least near the soil surface where alkaloids could have been temporarily unaltered. Alkaloids passing through the soil profile and associated food web system would have eventually led to their decomposition. Little is known about the fate of alkaloids in soil, and this topic warrants further investigation.

Net N mineralization was lower under tall fescue with low endophyte infection compared with high endophyte infection at a depth of 25 to 75 mm only (Fig. 6). A similar trend was observed at a depth of 0 to 25 mm. The difference in response to endophyte infection between C and N mineralization is curious, but not readily explainable. It may be that a greater amount of N was immobilized by microorganisms with low endophyte infection compared with high endophyte infection, especially near the soil surface. Various alkaloids produced by the endophyte–tall fescue association contain N within compound ring structures, which are relatively unaltered during passage through the grazing animal (Hill et al., 1994). With high endophyte infection, these N-containing compounds may have accumulated in various stages of decomposition during long-term pasture development and led to greater net N mineralization during incubation.

Ratios of basal soil respiration/soil microbial biomass C, basal soil respiration/soil organic C (data not shown),

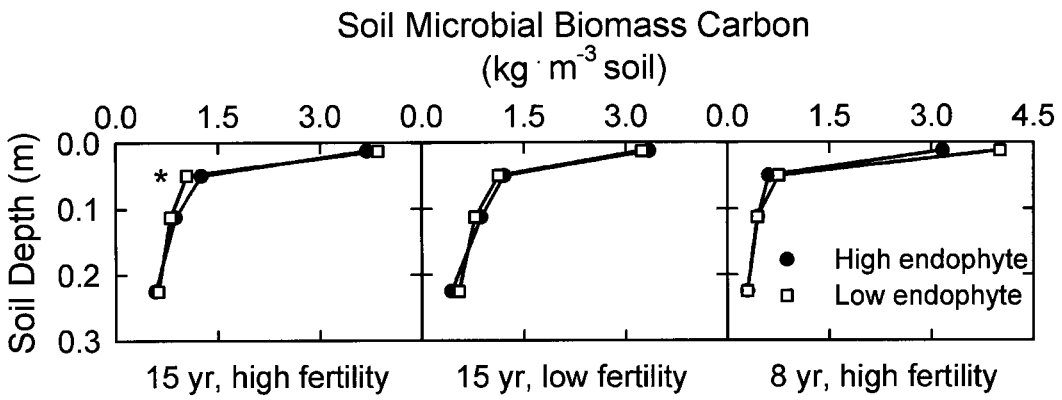

Fig. 5. Soil microbial biomass C with depth under tall fescue as affected by endophyte infection level; (* indicates significance at $P \leq 0.05$).

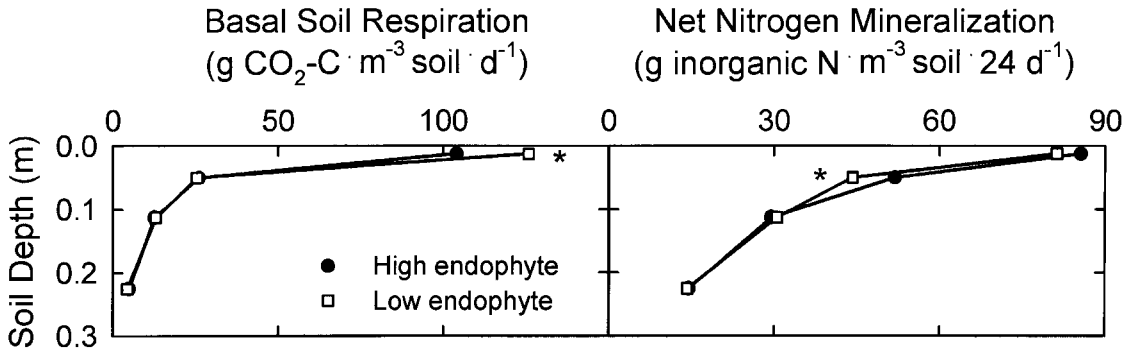

Fig. 6. Basal soil respiration and net N mineralization with depth under tall fescue as affected by endophyte infection level; (* indicates significance at $P \leq 0.05$).

soil microbial biomass C/particulate organic C, and basal soil respiration/particulate organic C (Fig. 7) at a depth of 0 to 25 mm were all greater under tall fescue with low endophyte infection compared with high endophyte infection. Ratios of basal soil respiration/particulate organic C, and soil microbial biomass C/particulate organic C, were the soil properties most sensitive to endophyte levels throughout the sampling depths (Fig. 7). These ratios reflect the combined effects of lower particulate organic C and greater microbial biomass and potential activity with low endophyte infection compared with high endophyte infection. Accumulation of more particulate organic matter with high endophyte infection, especially root-derived fragments, may have been due to enhanced plant growth or reduced herbage use by grazing animals. Lack of a proportional increase in microbial biomass and even a decrease in microbial activity suggests microbial inhibition in the presence of the endophyte.

Microbial Community Structure

We detected 100 fatty acid methyl ester peaks with C chain lengths ranging from 10 to 20. Of those 100 peaks, 79 had higher concentration ($P \leq 0.1$) at a depth of 0 to 25 mm than at a depth of 25 to 300 mm, and 45 had higher concentration at a depth of 25 to 75 mm than at a depth of 75 to 300 mm. In general, fatty acid methyl ester concentrations reflected the decrease in soil microbial biomass with depth, as determined with the chloroform fumigation-incubation method. Similarly, Zelles et al. (1995) reported a strong relationship between concentrations of ester-linked, phospholipid fatty acids and microbial biomass determined with substrate-induced respiration.

No significant change in the total number of fatty acids was observed between soils under tall fescue with 0 and 100% endophyte infection. An average of 65, 46, 40, and 27 peaks out of 100 were detected at depths of 0 to 25, 25 to 75, 75 to 150, and 150 to 300 mm, respectively. Only 68 to 86% of all peaks within a sample were matched with identities using the EUKARY method of the MIDI fatty acid methyl ester library, which was developed from phospholipid fatty acid profiles specific to soil organisms. Some of the unknown fatty acids from whole soils may have been derived from plant material and soil organic matter.

Concentrations of fatty acid methyl esters were mostly similar between soils under tall fescue with 0 and 100% endophyte infection. More frequently however, specific fatty acid methyl ester concentrations were lower under low than under high endophyte infection, particularly at a depth of 25 to 75 mm (Table 2). A wide range of alkaloids are produced in the endophytic association with tall fescue (Hill et al., 1994). These alkaloids may have had a small, direct impact on the microbial community structure by providing unique substrates during decomposition, but may have also shifted community dynamics to allow specific microbial groups to participate in various soil biological functions. None of the following signature fatty acids, however, differed significantly between endophyte infection levels: 18:2ω6 as fungal indicator (Vestal and White, 1989), 15:0 and

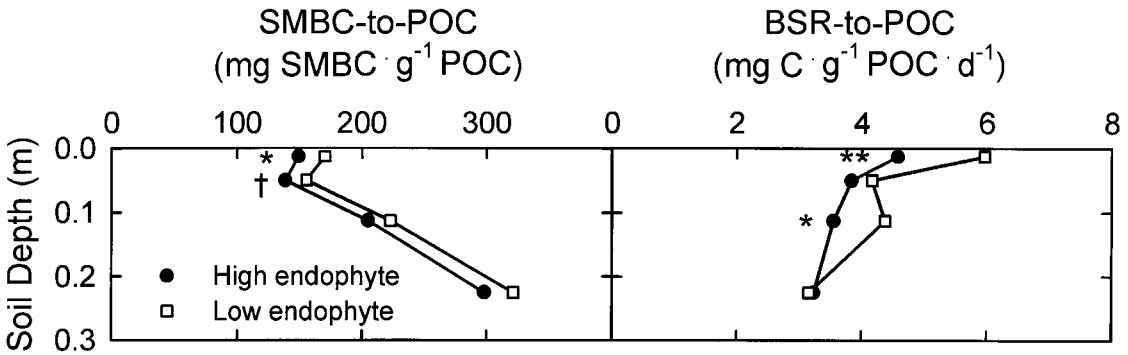

Fig. 7. Ratios of soil microbial biomass C (SMBC)/particulate organic C (POC) and basal soil respiration (BSR)/particulate organic C with depth under tall fescue as affected by endophyte infection level; (†, *, and ** indicate significance at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively).

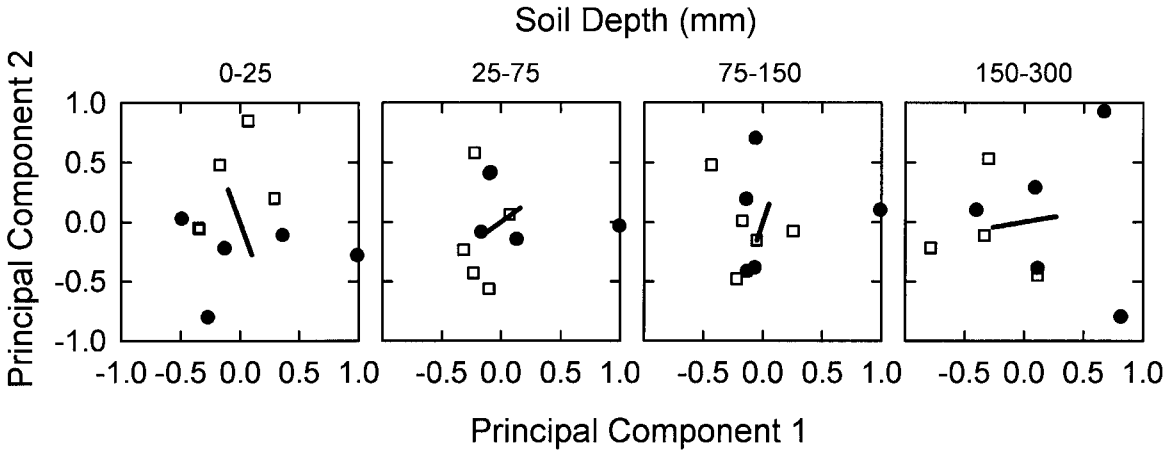

Fig. 8. Correlation biplots from principal component analysis of fatty acid methyl esters as affected by endophyte infection level within each soil depth. □ is low endophyte and ● is high endophyte. Length and orientation of lines indicate strength of each principal component.

17:0 as general indicators of bacteria (Vestal and White, 1989; Tunlid and White, 1992), iso and anteiso 15:0 as Gram-positive bacterial indicator (O'Leary and Wilkinson, 1988), and cy17:0 and cy19:0 as anaerobic bacterial indicator (Vestal and White, 1989). Depth distribution of these seven signature fatty acids was relatively similar with 53 ± 6% within the 0- to 25-mm depth, 25 ± 3% within the 25- to 75-mm depth, 16 ± 2% within the 75- to 150-mm depth, and 6 ± 3% within the 150- to 300-mm depth.

When whole-soil fatty acid methyl ester data were subjected to principal components analysis, separation between soils under tall fescue with 0 and 100% endophyte infection was observed along the second principal component at a depth of 0 to 25 mm and along the first principal component at a depth of 150 to 300 mm (Fig. 8). No readily apparent differences were observed at other depths. These results suggest that soil microbial communities between the two fescue systems were somewhat different in the residue-enriched surface layer (0–25 mm), where alkaloid-affected plant litter, dung, and urine were deposited, but more similar at a depth of 25 to 300 mm. The small difference in the microbial community structure between soils under fescue with 0 and 100% endophyte infection may have been a consequence of endophyte byproducts that limited heterotrophic activity or allowed other organisms to develop a niche in this soil ecosystem. Further work is needed to ascertain whether the differences in microbial activity and community structure were due indirectly to differences in plant productivity or due directly to positive or negative feedbacks of endophyte infection on heterotrophic activity and community structure.

Table 2. List of significant changes in individual fatty acid methyl ester concentrations by soil depth, due to the presence of the endophyte (*Neotyphodium coenophialum*) in tall fescue.

ECL†	Compound	Trt>‡
0–25 mm depth		
10.00	10:0	E−§
14.28	?¶	E−
14.53	C15 N alcohol	E+
14.92	?	E+
17.24	16:0 2OH	E+
17.26	C14 dicarboxilic	E−
17.52	16:0 3OH	E+
18.59	19:0 N alcohol	E+
18.75	19:1 ω11c	E+
25–75 mm depth		
13.73	?	E+#
13.90	14:1 ω5c	E+
15.55	C16 N alcohol	E−
15.70	?	E−
15.73	16:2 ω6c	E+
16.36	?	E+
16.52	17:1 anteiso ω9c	E+
17.45	18:1 iso H	E+
17.54	?	E+
17.77	18:1 ω9c	E+
18.00	18:0	E+
18.32	?	E+
18.44	?	E+
18.59	19:0 N alcohol	E+
19.09	18:1 2OH	E+
19.17	?	E+
19.22	?	E+
19.44	?	E+
19.60	C20 N alcohol	E+
75–150 cm depth		
13.94	11:1 2OH	E−
16.48	17:1 iso I/anteiso B	E+
150–300 mm depth		
12.10	?	E−
13.04	?	E−
14.06	?	E−
15.55	C16 N alcohol	E−
17.47	17:0 dimethyl acetal	E−

† ECL is equivalent chain length.
‡ Trt> indicates the endophyte level with greater concentration.
§ E− is 0% infection.
¶ ? symbol denotes an unidentified compound in the MIDI library.
# E+ is 100% infection.

SUMMARY AND CONCLUSIONS

We conclude that high endophyte infection of tall fescue, despite its negative impacts on animal performance and productivity, contributes to greater soil C sequestration in soils of the Southern Piedmont USA. The difference in soil organic C storage in the 0- to 300-mm depth under tall fescue with low and high endophyte infection was 0.18 ± 0.20 kg m^{-2} (mean ± SD among experimental sets). These differences approach the increase in soil organic C upon conversion from conventional-tillage to no-tillage crop production in Georgia

(0.23 ± 0.15 kg m^{-2} [Franzluebbers et al., 1999b]; 0.46 kg m^{-2} [Beare et al., 1994]). Greater microbial activity may have been at least partially responsible for the lower accumulation of soil organic C and total N with low endophyte infection compared with high endophyte infection. Further, some evidence of the altered microbial community structure in response to endophyte infection was observed at the soil surface.

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