

Soil microbial activity and diversity under *Festuca arundinacea* infected with *Neotyphodium coenophialum*

L'activité et la diversité des microbes du sol sous *Festuca arundinacea* infectée par *Neotyphodium coenophialum*

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Rationale

Tall fescue (*Festuca arundinacea*) 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 14 million ha 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). 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 with high endophyte level ingested 65-92% less forage, produced 57-83% less milk, gained 21-78% less weight per day, and gained 65-89% less weight per hectare compared with animals fed tall fescue with low endophyte level (Stuedemann and Hoveland, 1988). Reduced animal production and performance is related to the presence of toxic ergopeptine alkaloids that accumulate in endophyte-infected tall fescue (Stuedemann and Thompson, 1993). These alkaloids may also protect the plant from insect herbivory. Endophyte-free perennial ryegrass (*Lolium perenne*), which also normally benefits from an endophytic association in nature, is rapidly consumed by herbivorous insects (Prestidge et al., 1982; Latch,

1993; Rowan and Latch, 1994) due to the lack of alkaloids that would normally deter such activity.

In addition to biochemical deterrents, *N. coenophialum* may confer drought tolerance to tall fescue (Bouton et al., 1993; West et al., 1993). Suggested mechanisms for drought resistance in endophyte-infected compared with endophyte-free tall fescue are lower net photosynthetic rate and higher stomatal resistance (Belesky et al., 1987) and greater root proliferation under drought-stressed conditions (Richardson et al., 1990).

Because toxic alkaloids in endophyte-infected tall fescue may deter foraging cattle, herbivorous insects, pathogenic fungi, viruses, and root-feeding nematodes (Latch, 1997), we hypothesized that accumulation of these same alkaloids in plant litter, dung, urine, and soil may alter soil organic matter dynamics and microbial diversity compared with endophyte-free tall fescue. Our objectives were to characterize (i) the size of soil organic C and N pools, (ii) potential microbial activity, and (iii) microbial diversity under endophyte-free tall fescue compared with endophyte-infected tall fescue.

Methodology

Two replicate paddocks (0.8 ha) were planted to endophyte-infected Kentucky-31 tall fescue (E+) in autumn 1987 and two paddocks were planted to endophyte-free tall fescue (E-) from the same seed source in autumn 1988 near Watkinsville, GA (33° 62' N, 83° 25' W) on Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludults). Paddocks were grazed with Black Angus cattle each year thereafter.

Soil samples were collected in January 1997 at depths of 0-2.5, 2.5-7.5, 7.5-15, and 15-30 cm at distances of 1, 10, 30, 50, and 80 m from permanent shade and water sources, which were located \approx 20 m apart along one edge of each paddock. Eight cores (4.1-cm diam) were composited from each depth and distance. Analyses were conducted from each depth separately, but we present the standing stock of soil properties representing the 0 to 30 cm depth only. Soil was oven-dried (55 °C, 48 hr), weighed, and crushed to pass a screen (4.75-mm openings) to partially homogenize sample and remove stones (<1% of weight). Bulk density was calculated from the oven-dried weight of soil and volume of coring device. A subsample was ground to a fine powder and analyzed for total C and N with dry combustion. It was assumed that total C was equivalent to organic C, because soil pH was near 6.

Two subsamples of soil (15-60 g each) were wetted to 50% water-filled pore space, placed into a 1-L canning jar along with vials containing 10 mL of 1 M NaOH to trap evolved CO₂ and water to maintain humidity, and incubated at 25 °C for 24 d (Franzluebbers and Arshad, 1996). Alkali traps were replaced at 3 and 10 d. Evolved CO₂ 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 CHCl₃, 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 CO₂ evolved from the fumigated sample during 10 d divided by an efficiency factor of 0.41 (Voroney and Paul, 1984).

Particulate organic C and N were determined by shaking 15-60 g of soil with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ for 16 hours, collecting the sand plus organic matter retained on a 0.06 mm screen, oven-drying, weighing, grinding, and determining the C and N concentration using dry combustion (Cambardella and Elliott, 1992).

Soil microbial diversity was determined from whole soil fatty acid methyl esters (Cavigelli et al., 1995). Fatty acid methyl esters 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 x g for 20 min, collection of two-thirds of the hexane layer in a test tube, and incubation 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 and analyzed with an HP 5890 gas-liquid chromatograph (Hewlett Packard, Rolling Meadows, IL) equipped with an HP Ultra 2 capillary column (crosslinked 5% phenyl methyl silicone, 25 m by 0.2 mm) and a flame ionization detector. Whole soil fatty acid methyl ester profiles were compared using principal component analysis.

Pair-wise t-tests were used to test the significance between endophyte treatments of soil properties across depths as a standing stock value using SAS. Pairs were blocked according to replicate paddock and distance from shade and water sources. Significance has been denoted at $P \leq 0.1$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***) in tables.

Results and discussion

Soil bulk density to a depth of 30 cm was unaffected by the removal of the endophyte from tall fescue (Table 1). Soil organic C and N, individually or as a ratio, were also unaffected by the endophyte.

Particulate organic C tended ($P=0.13$) to be lower and particulate organic N was significantly lower under E- than under E+ to a depth of 30 cm (Table 1). Less particulate organic C and N under E- than under E+ could be an indication of less root proliferation of tall fescue, as this fraction expresses the contribution of roots more than total organic C and N. Less vigorous rooting under E- compared with E+ has been previously suggested (Richardson et al., 1990). In a rhizotron study, Knox (1994) observed reduced root length under E- to a depth of 100 cm beginning four weeks after sprigging compared with E+ during a year with cooler temperature, but no difference between E- and E+ in a year with hotter temperature that produced only 15-20% of the total root length in the cooler year.

Basal soil respiration was greater under E- than under E+ to a depth of 30 cm (Table 1). This result indicates that toxic alkaloids from endophyte infection may have inhibited microbial activity. However, little is known about the fate of alkaloids in soil and this warrants further investigation.

Soil microbial biomass C was also greater under E- than under E+ to a depth of 30 cm (Table 1). If toxic alkaloids resulting from endophyte infection of tall fescue were responsible for reduced soil microbial biomass and activity, then these alkaloids had a greater effect on microbial activity than biomass at a depth of 0-2.5 cm, but a greater effect on microbial biomass than activity at a depth of 2.5-7.5 cm (data not shown). The

ratio of basal soil respiration-to-soil microbial biomass C, however, was unaffected by the presence of the endophyte to a depth of 30 cm (Table 1).

The ratios of basal soil respiration-to-soil organic C and soil microbial biomass C-to-soil organic C indicated similar effects with respect to the presence of the endophyte on absolute quantities of these pools (Table 1). The ratio of soil microbial biomass C-to-particulate organic C was also greater under E- than under E+. This result indicates that more coarse organic material (particularly roots) may have accumulated due to enhanced plant growth without a proportional increase in microbial biomass due to microbial inhibition in the presence of the endophyte.

Table 1. Standing stock of soil C and N pools to a depth of 30 cm under endophyte-free (E-) and endophyte-infected (E+) tall fescue.

Soil property	E-	E+	P>F	CV (%)
Bulk density ($\text{Mg} \cdot \text{m}^{-3}$)	1.36	1.34	NS	3
Soil organic C, SOC ($\text{kg} \cdot \text{m}^{-2}$)	3.28	3.29	NS	12
Soil organic N, SON ($\text{kg} \cdot \text{m}^{-2}$)	0.21	0.22	NS	11
Soil organic C-to-N ($\text{g} \cdot \text{g}^{-1}$)	15.4	15.1	NS	3
Particulate organic C, POC ($\text{kg} \cdot \text{m}^{-2}$)	1.08	1.19	NS	13
Particulate organic N, PON ($\text{kg} \cdot \text{m}^{-2}$)	0.04	0.05	*	18
Particulate organic C-to-N ($\text{g} \cdot \text{g}^{-1}$)	26.6	24.9	NS	11
POC-to-SOC ($\text{g} \cdot \text{g}^{-1}$)	0.33	0.36	NS	13
PON-to-SON ($\text{g} \cdot \text{g}^{-1}$)	0.19	0.22	*	14
Soil microbial biomass C, SMBC ($\text{g} \cdot \text{m}^{-2}$)	218	190	*	10
SMBC-to-SOC ($\text{mg} \cdot \text{g}^{-1}$)	67.5	58.1	*	16
SMBC-to-POC ($\text{mg} \cdot \text{g}^{-1}$)	203	160	**	12
Basal soil respiration, BSR ($\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$)	6.20	5.01	*	16
BSR-to-SOC ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$)	1.93	1.52	*	20
BSR-to-POC ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$)	5.75	4.18	***	11
BSR-to-SMBC ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$)	28.3	26.2	NS	10

We detected 100 fatty acid methyl ester peaks with C chains ranging from 10 to 20. Of those 100 peaks, 79 were more concentrated at a depth of 0-2.5 cm compared with a depth of 2.5-30 cm and 45 were more concentrated at a depth of 2.5-7.5 cm compared with a depth of 7.5-30 cm. In general, fatty acid concentrations reflected the decrease in soil microbial biomass with depth, as determined with the chloroform fumigation-incubation method. Zelles et al. (1995) reported a strong relationship between concentrations of ester-linked, phospholipid fatty acids and microbial biomass determined with substrate-induced respiration.

More frequently, specific fatty acid methyl ester concentrations were lower under E- than under E+, particularly at a depth of 2.5-7.5 cm (Table 2). There was no significant change in the number of fatty acids between endophyte levels, with an average of 65, 46, 40, and 27 peaks out of a total of 100 detected at depths of 0-2.5, 2.5-7.5,

7.5-15, and 15-30 cm, respectively. The MIDI fatty acid methyl ester library was able to identify 68-86% of all peaks. There is a wide range of alkaloids produced in the endophytic association with tall fescue (Hill et al., 1994). These alkaloids may have had a small, direct impact on microbial diversity by providing unique substrates during decomposition, but may have also indirectly shifted community dynamics to allow new microbial groups to participate in various soil biological functions.

Table 2. List of significant changes in individual fatty acid methyl ester concentrations due to the presence of the endophyte in tall fescue. Trt > indicates the endophyte level with greater concentration.

Peak	Compound	Trt >	Peak	Compound	Trt >
<i>0-2.5 cm depth</i>			<i>2.5-7.5 cm depth</i>		
10.00	10:0	E-	13.73	?	E+
14.28	?	E-	13.90	14:1 ω 5c	E+
14.53	C15 N alcohol	E+	15.55	C16 N alcohol	E-
14.92	?	E+	15.70	?	E-
17.24	16:0 2OH	E+	15.73	16:2 ω 6c	E+
17.26	C14 dicarboxylic	E-	16.36	?	E+
17.52	16:0 3OH	E+	16.52	17:1 anteiso ω 9c	E+
18.59	19:0 N alcohol	E+	17.45	18:1 iso H	E+
18.75	19:1 ω 11c	E+	17.54	?	E+
			17.77	18:1 ω 9c	E+
			18.00	18:0	E+
<i>7.5-15 cm depth</i>			18.32	?	E+
13.94	11:1 2OH	E-	18.44	?	E+
16.48	17:1 iso I/anteiso B	E+	18.59	19:0 N alcohol	E+
			19.09	18:1 2OH	E+
<i>15-30 cm depth</i>			19.17	?	E+
12.10	?	E-	19.22	?	E+
13.04	?	E-	19.44	?	E+
14.06	?	E-	19.60	C20 N alcohol	E+
15.55	C16 N alcohol	E-			
17.47	17:0 dimethyl acetal	E-			

When whole-soil fatty acid methyl esters were subjected to principal component analysis, no difference between E- and E+ was observed. This result suggests that the diversity of soil microbial communities in the rhizosphere of the two fescues was similar. It appears that any long-term, potential difference in microbial diversity between E- and E+ was small at best.

Summary

At the end of eight years of cattle grazing on endophyte-free tall fescue, total and passive soil C pools (i.e., soil organic C and particulate organic C) were similar or lower,

but active soil C pools (i.e., soil microbial biomass C and basal soil respiration) were greater compared with grazing on endophyte-infected tall fescue. Inhibition of microbial growth and activity in the presence of toxic alkaloids may have led to these differences. Only small changes were observed in microbial diversity with the removal of the endophyte from tall fescue.

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