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Short-term responses of soil C and N fractions to tall fescue endophyte infection

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Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is naturally infected with a fungal endophyte, *Neotyphodium coenophialum*, which produces toxic ergot alkaloids that negatively affect herbivores and that may alter soil organic matter dynamics. A 60-week mesocosm study with a factorial arrangement of soil type (clay loam and loamy sand) and endophyte infection (with and without) was conducted to determine potential changes in soil C and N fractions. Forage and root dry-matter production were greater with than without endophyte infection, while forage C and N concentrations were unaffected. Total, particulate, mineralizable, and aggregate-associated C and N fractions increased several fold during the course of the experiment due to large rhizosphere inputs in all treatments. The fraction of total C and N in water-stable macroaggregates (>0.25 mm) was initially 0.43 ± 0.10 and 0.46 ± 0.16, respectively, and increased during the course of the experiment to 0.68 ± 0.06 and 0.56 ± 0.15 when averaged across soil type and endophyte infection level as a result of organic matter cycling and deposition in this active biophysical fraction. Changes in soil C and N fractions due to endophyte infection were minimal. The lack of detectable changes in soil C and N fractions due to endophyte infection may have been a result of the overwhelming input of C from roots and/or the relatively short-term nature (60 weeks). Greater plant productivity of endophyte-infected tall fescue is likely a contributing mechanism for eventual changes in total and active C and N fractions that have been observed in long-term pastures.

Abbreviations: E− – endophyte free; E+ – endophyte infected

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is an important grass grown around the world for forage and turf and is considered the most important perennial, cool-season grass for cattle production in the southeastern USA. It is naturally infected with a fungal endophyte, *Neotyphodium coenophialum*, which resides in the above-ground portions of the plant and produces a variety of alkaloids that have been shown to be toxic when consumed in large quantities by grazing cattle, sheep, and horses (Stuedemann and Hoveland, 1988). Endophyte-free (E−) tall fescue pastures can be developed by planting seed that has had the fungus killed during prolonged storage. However, endophyte-free pastures have not been widely developed for long-term grazing systems, because stands are not persistent due to overgrazing and lower disease and pest resistance (Hoveland, 1993). The funal endophyte of tall fescue (E+), therefore, is considered an important component in the agroecological fitness of tall fescue (Clay, 1997).

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One of the positive ecological consequences of endophyte infection can be found in the soil. Total soil organic C and N contents were found to be greater under high- than under low-endophyte-infected pastures (Franzluebbers et al., 1999). One reason for the difference in soil organic C and N could be related to greater fitness of E+ tall fescue, in which seedling dry matter production can be greater than under E− tall fescue (Cheplick et al., 1989; Clay, 1993). Greater plant production capability could lead to greater C input to soil resulting in greater soil organic C. Another reason for the difference in soil organic C and N contents could be due to altered soil microbial dynamics. Per unit of soil organic C, potential C mineralization was lower under high- than under low-endophyte-infected tall fescue pastures that were 8–15 years old (Franzluebbers et al., 1999). Further, E+ ryegrass (*Lolium multiflorum* Lam.) leaf litter decomposed slower than E− leaf litter in a 12-week outdoor microcosm (Omacini et al., 2004). During a 32-day decomposition experiment, C mineralization and microbial biomass C were slightly inhibited by E+ tall fescue leaves compared with E− leaves, but net N mineralization and microbial biomass N were enhanced by endophyte infection (Franzluebbers and Hill, 2005). Both greater C input and altered microbial processing of leaf litter appear to be possible mechanisms for greater soil organic C and N accumulation in long-term E+ than E− tall fescue pastures. Further research is needed to understand the dynamics and pathways of how soil organic C and N can be affected by the tall fescue-endophyte association, where both short-term soil biological alterations and long-term soil C and N changes have been observed.

Plant residues returned to the soil are exposed to a variety of decomposition steps and processes, contributing to a gradient in soil biochemical potential from active to passive (Paul and Clark, 1996). Microbial biomass and mineralizable C and N are active components of soil that can be used to characterize biochemical availability of organic resources in soil (Wander, 2004). One of the intermediate stages of decomposition can be characterized with the physical separation of soil as particulate organic matter (Cambardella and Elliott, 1992). Particulate organic C was greater under long-term tall fescue pastures with high than with low endophyte infection (Franzluebbers and Stuedemann, 2002), suggesting that reduced decomposition is possible due to exposure of soil to endophyte-infected tall fescue. Another physical fraction of soil, in which significant storage of soil organic C and N can occur, is water-stable macroaggregates (Tisdall and Oades, 1982). In long-term tall fescue pastures, greater storage of soil organic C and N due to high endophyte infection was found preferentially in water-stable macroaggregates compared with microaggregates (Franzluebbers and Stuedemann, 2005). Organic matter stored in macroaggregates can become physically protected from decomposition by isolating organic matter resources from the activity of soil fauna and microorganisms (Beare et al., 1994; Elliott, 1986).

A mesocosm experiment was conducted to determine the fraction of soil most susceptible to change in response to short-term exposure of soil to E+ and E− tall fescue plants. Should aggragate-associated soil be one of the fractions most affected by endophyte infection, two soil types with different particle-size distributions were evaluated to discern the influence of particle size on changes in aggregate dynamics. A semi-controlled experiment with multiple evaluations of soil was employed to overcome large inherent spatial variability in soil properties in typical pastures and to help discern temporal changes. The working hypothesis was that E+ tall fescue would inhibit soil biological activity throughout the experiment and eventually lead to enhanced organic C and N compared with E- tall fescue.

Materials and methods

Experimental setup

An outdoor mesocosm study consisting of 48 experimental units was conducted from March 2002 until April 2003 near Watkinsville GA (33°52′ N, 83°25′ W). Climatic conditions during the experiment are shown in Figure 1. The experimental design consisted of three randomized replications of a factorial arrangement of soil type (clay loam and loamy sand) and endophyte infection (E− and E+) placed in four blocks that were sequentially harvested at 8, 20, 36, and 60 weeks of growth. Particle size distribution in the clay loam was 325 ± 5 mg g^{-1} clay, 213 ± 2 mg g^{-1} silt,

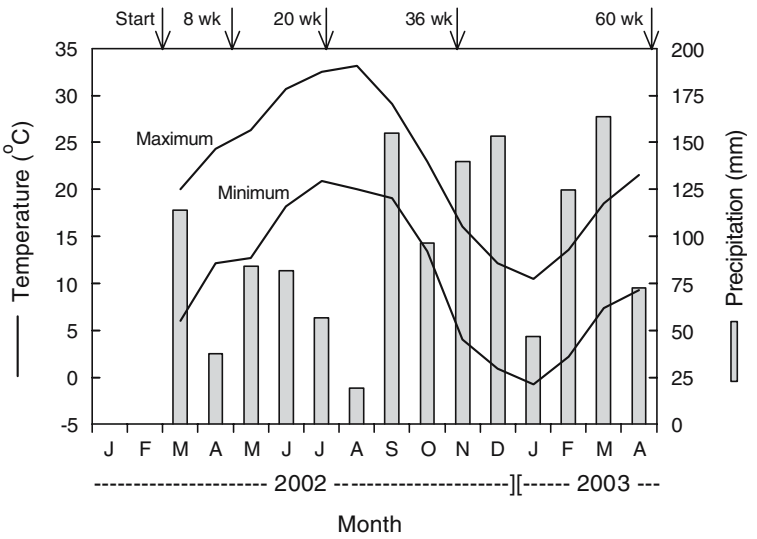

Figure 1. Mean monthly air temperature (based on minimum and maximum values for each day) and precipitation in Watkinsville GA during the course of the 60-week incubation.

and 463 ± 3 mg g^{-1} sand and in the loamy sand was 240 ± 3 mg g^{-1} clay, 125 ± 14 mg g^{-1} silt, and 635 ± 12 mg g^{-1} sand. Soil was collected locally from a field dominated by Cecil-Pacolet-Appling series (clayey, kaolinitic, thermic Typic Kanhapludults) at an excavated site representing subsoil at ~1-m depth (clay loam) and at a drainage way representing alluvial wash (loamy sand).

Containers were 15 cm in diameter and height, enclosed at the bottom with a double layer of vinyl cloth with 1-mm openings, and placed on a wooden frame covered with hardware wire mesh to allow air to desiccate roots that reached the bottom. Tillers of 2- to 3-year-old 'Jesup' tall fescue plants were excavated the morning of the start of the experiment (5 March 2002) from two adjacent pastures containing E− and E+ and washed free of soil. Endophyte-free pastures were established with seed that did not contain the endophytic fungus by subjecting it to long-term storage conditions. Ergot alkaloid concentration of forage harvested at the end of the experiment was below detection limit under E− in both soils and 16.7 ± 0.8 μg g^{-1} under E+ (no difference between soils), which verified the absence and presence of the endophyte. Five tillers were placed in 2.5 kg of the clay loam and 2.7 kg of the loamy sand, which represented initial bulk density of 1.1 and 1.2 Mg m^{-3}, respectively. Containers were irrigated (1) twice weekly with 0.4 L pot^{-1} with a nutrient solution containing 7.5 mg NH_4–N, 7.5 mg NO_3–N, 35 mg urea–N, 50 mg P_2O_5, 50 mg K_2O, 0.5 mg chelated Fe, 0.25 mg chelated Mn, 0.25 mg chelated Zn, and 1.25 mg humic acid L^{-1} and (2) when needed with tap water to avoid desiccation. From December 2002 to February 2003, containers were moved into an unheated greenhouse to avoid complete dormancy during the winter. All containers remaining in the experiment received the same amount of supplemental irrigation, which varied depending upon rainfall and temperature throughout the year.

At 8, 20, and 36 weeks of growth, those experimental units not removed for plant and soil analyses had the forage clipped ~3 cm above the soil and placed on the soil surface to decompose. This protocol allowed some return of above-ground plant material to the surface, but did not mimic natural pasture dynamics closely where grazing and dung deposition are the major pathway and ungrazed plant material senesces without clipping prematurely.

Plant and soil sampling and analyses

At each of the four sampling dates, 12 experimental units were removed for analysis. Above-ground plant matter was clipped at the soil surface, chopped into 2- to 3-cm long segments, mixed, and divided into two portions: (1) a 5-g subsample dried

at 55 °C for 48 h, weighed, ground to < 1 mm, and analyzed for total C and N concentration with dry combustion and (2) the remaining sample stored for a few days at 4 °C until freeze-dried, then extracted for ergot alkaloid determination with an enzyme-linked immunosorbance assay (Hiatt and Hill, 1999). Soil was separated from roots by hand-working contents over a screen with 8-mm openings. Roots were washed, dried at 55 °C for 3 days, and weighed. Soil was separated into two subsamples: (1) kept field-moist at 4 °C until microbiological analyses could be conducted (not reported here) and (2) dried at 55 °C for 3 days and passed through a screen with 4.75-mm openings prior to all analyses reported here.

Total organic C and N of soil were determined by dry combustion (Leco Tru-Spec)[1] following ball-milling of a subsample to a fine powder for 5 min. Particulate organic C and N were determined by dry combustion following ball-milling of the dried (55 °C, 72 h), sand-sized fraction (> 0.053 mm) obtained from a soil subsample (65 g) dispersed in 100 mL of 0.01 *M* $Na_4P_2O_7$ by shaking overnight (Cambardella and Elliott, 1992; Franzluebbers et al., 1999). Mineralizable C and N were determined from the CO_2–C trapped in an alkali trap and inorganic N accumulated during incubation of soil at 25 °C for 24 days. Two soil subsamples (65-g each) were wetted to 50% water-filled pore space in graduated bottles and placed in a 1-L sealed, canning jar along with a vial of 10 mL of 1 *M* NaOH to trap CO_2 and a vial of water to maintain humidity during incubation. Alkali traps were replaced at 3 and 10 days. At 10 days, one of the subsamples was removed, fumigated with $CHCl_3$ for 24 h, vapors removed, and placed in a separate canning jar along with an alkali trap for 10 days. Soil microbial biomass C was calculated from the CO_2–C released during incubation of the fumigated sample without subtraction of a control (Franzluebbers et al., 1999; Voroney and Paul, 1984). Inorganic N was determined at 0 and 24 days from a filtered extract of a 10-g subsample of dried (55 °C, 48 h) and sieved (< 2 mm) soil shaken with 20 mL of 2 *M* KCl for 20 min using salicylate-nitroprusside and Cd-reduction autoanalyzer techniques (Bundy and Meisinger, 1994).

Dry aggregate distribution was determined by placing a 100-g subsample of soil on top of a nest of sieves (20-cm diam), shaking for 1 min at Level 6 on a CSC Scientific Sieve Shaker (Catalogue No. 18480), and weighing soil retained on 1.0-, 0.25-, and 0.05-mm screens and that passing the 0.05-mm screen (Kemper and Koch, 1966). Water-stable aggregate distribution was determined from the reconstituted sample used for dry aggregate distribution placed on top of a nest of sieves (17.5-cm 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}). After removing the two sieves and placing them in an oven to dry, water containing soil passing the 0.25-mm sieve was poured over a 0.05-mm sieve, soil washed with a gentle stream of water, and the soil retained transferred into a drying bottle with a small stream of water. The < 0.05-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 3 days and a subsample analyzed for total C and N concentration with dry combustion. The > 1-mm fraction was ball-milled prior to C and N determination. 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. Stability of mean-weight diameter was calculated as water-stable mean-weight diameter divided by dry-stable mean-weight diameter. Macroaggregates from both dry and wet sieving procedures were defined as > 0.25 mm. Stability of macroaggregates was calculated as water-stable macroaggregates divided by dry-stable macroaggregates.

Statistical analyses

Endophyte treatment effects within a sampling period and soil type were considered significant at $P \leq 0.1$. Since many plant and soil properties changed dramatically with increasing growth stage, linear regression with time (i.e., single degree of freedom) was used to determine if the effects of soil type, endophyte, and soil type x endophyte were altered with time.

Results

Cumulative dry matter production of tall fescue forage under these well-irrigated and well-fertil-

ized conditions reached an average of 62 g pot^{-1} by the end of 60 weeks of growth (Figure 2). This was equivalent on an area basis to 35 Mg ha^{-1}, which would be several times greater than expected under normal field conditions in this region. Under these ideal conditions, soil type had little influence on above- or below-ground dry matter production, but endophyte infection did (Table 1). With increasing time, both above- and below-ground dry matter production became greater with E+ than with E- (Figure 2).

Forage C and N concentrations were only affected by sampling period (Table 1), in which C concentration and C:N ratio increased with time and N concentration decreased with time (Figure 3). Neither soil type nor endophyte infection had an effect on forage C and N concentration.

Total soil organic C and N concentrations were affected primarily by soil type and sampling period (Table 1). In both soil types, total organic C increased by ~3 mg g^{-1} during 60 weeks, which nearly doubled the C concentration in the loamy sand and more than tripled the C concentration in the clay loam (Figure 4). Total soil C:N ratio varied relatively little with time in the loamy sand, but increased with time in the clay loam. Although strong endophyte effects were not apparent in the overall analysis of total C and N (Table 1), there were occasional differences due to endophyte infection in a particular soil on a particular sampling date (Figure 4). These included lower total organic C concentration under E+ than E− in the clay loam at 36 and 60 weeks, lower total N concentration under E+ than E− in the loamy sand at 36 weeks, and higher total N concentration under E+ than E− in the clay loam at 8 weeks.

Particulate organic C and N concentrations were mostly affected by sampling period, but were also affected by soil type and endophyte infection (Table 1). Like total C concentration, particulate organic C increased dramatically with time in both soils (Figure 4). Particulate organic N increased with time, but relatively less than particulate organic C. The increase in particulate organic C with time was greater in the clay loam than in the loamy sand, both on a relative basis (27 vs. 2 times greater) and an absolute basis (1.5 vs. 1.3 mg g^{-1} greater). The increase in particulate organic C with time was also lower under E+ than under E−, when averaged across soil types (1.3 vs. 1.5 mg g^{-1}). Averaged across sampling periods, there was a significant interaction between soil type and endophyte infection for particulate organic N, in which E+ was relatively greater than under E− in the clay loam

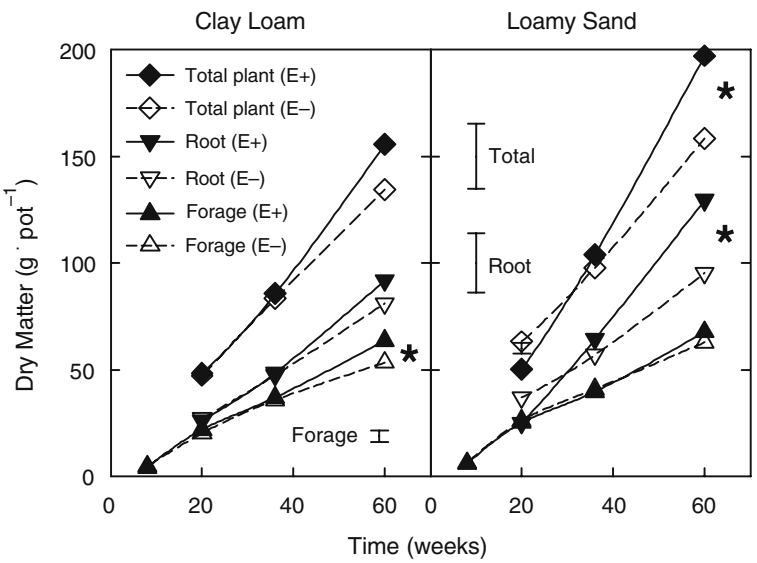

Figure 2. Cumulative dry matter production of forage, roots, and total plant as affected by endophyte infection (E− is endophyte-free, E+ is endophyte-infected) in two soils harvested at different times during a 60-week growth period. * indicates difference between endophyte infection means within a soil type and sampling period at $P=0.1$. Error bars denote least significant difference among all means (sampling period, soil type, and endophyte infection) within a plant-part component at $P=0.1$.

Table 1. Analysis of variance of plant and soil properties in response to soil type (clay loam and loamy sand), endophyte infection (without and with), sampling period (linear regression of 8, 20, 36, and 60 weeks), and their interactions

Response variable	R^2	Coefficient of variation (%)	Soil Type (ST)	Endophyte (Endo)	ST×Endo	Sampling Period (SP)	ST×SP	Endo×SP	ST×Endo×SP
			Pr > F						
Cumulative forage mass (g pot^{-1})	0.97	13	0.50	0.24	0.92	**< 0.001**	0.18	**0.02**	0.43
Cumulative root mass (g pot^{-1})	0.79	30	0.68	0.19	0.41	**< 0.001**	0.16	**0.06**	0.27
Cumulative total plant mass (g pot^{-1})	0.87	20	0.83	0.14	0.44	**< 0.001**	0.16	**0.03**	0.35
Forage C (mg C g^{-1} forage)	0.28	1	0.83	0.78	0.80	**< 0.001**	0.73	0.92	0.77
Forage N (mg N g^{-1} forage)	0.89	11	0.80	0.68	0.70	**< 0.001**	0.96	0.93	0.77
Forage C:N ratio (g C g^{-1} N)	0.94	9	0.75	0.80	0.54	**< 0.001**	0.45	0.32	0.48
Total organic C (mg C g^{-1} soil)	0.95	11	**< 0.001**	0.86	0.82	**< 0.001**	0.19	0.43	0.37
Total N (mg N g^{-1} soil)	0.83	10	**< 0.001**	0.93	0.21	**< 0.001**	**0.07**	0.62	0.27
Total C:N ratio (g C g^{-1} N)	0.91	10	**< 0.001**	0.86	0.27	**< 0.001**	**< 0.001**	0.66	0.89
Particulate organic C (mg C g^{-1} soil)	0.96	11	**< 0.001**	0.18	0.81	**< 0.001**	**0.04**	**0.03**	0.75
Particulate organic N (mg N g^{-1} soil)	0.61	24	0.74	0.87	**0.02**	**< 0.001**	0.89	0.73	**0.10**
Particulate C: N ratio (g C g^{-1} N)	0.59	37	**< 0.001**	0.31	**0.09**	**0.03**	**0.03**	0.55	0.34
Microbial biomass C (μg C g^{-1} soil)	0.63	20	0.16	0.85	0.73	**< 0.001**	**0.05**	0.83	0.60
Mineralizable C (μg C g^{-1} soil 24 day^{-1})	0.68	34	0.21	0.81	0.83	**< 0.001**	0.15	0.58	0.86
Mineralizable N (μg N g^{-1} soil 24 day^{-1})	0.89	27	0.18	0.75	0.79	**< 0.001**	**< 0.001**	0.42	0.71
Mineralizable C:N ratio (g C g^{-1} N)	0.42	55	**0.003**	0.18	0.42	**0.002**	0.13	0.25	0.49
Water-stable macroaggregates (g g^{-1})	0.98	4	**< 0.001**	0.40	0.82	**0.02**	0.58	0.33	0.28
Macroaggregate stability (g$_{[wet]}$ g$^{-1}_{[dry]}$)	0.84	11	**< 0.001**	0.55	0.49	**< 0.001**	0.37	0.41	0.72
Mean-weight diameter of WSA (mm)	0.81	8	**< 0.001**	0.64	0.68	**0.02**	0.62	0.28	0.76
MWD[a] stability (mm$_{[wet]}$ mm$^{-1}_{[dry]}$)	0.47	20	**0.01**	0.58	0.50	**0.003**	0.97	0.31	0.57
C in >1-mm WSA[b] (mg C g^{-1} soil)	0.74	37	0.42	0.90	0.64	**< 0.001**	0.47	0.95	0.64
N in >1-mm WSA (mg N g^{-1} soil)	0.61	38	0.57	0.93	0.76	**< 0.001**	0.69	0.55	0.95
>1-mm WSA C:N ratio (g C g^{-1} N)	0.78	15	**0.02**	0.54	0.81	**< 0.001**	**0.05**	0.26	0.54
C in 0.25–1-mm WSA (mg C g^{-1} soil)	0.95	16	**< 0.001**	0.53	0.85	**< 0.001**	**0.002**	0.11	0.99
N in 0.25–1-mm WSA (mg N g^{-1} soil)	0.86	21	**< 0.001**	0.71	0.20	**< 0.001**	0.35	0.40	0.15
0.25–1-mm WSA C:N ratio (g C g^{-1} N)	0.72	21	**< 0.001**	0.76	0.12	**< 0.001**	**0.06**	0.40	**0.03**
C in 0.05–0.25-mm WSA (mg C g^{-1} soil)	0.86	15	**< 0.001**	0.68	0.74	**< 0.001**	**< 0.001**	0.21	0.30
N in 0.05–0.25-mm WSA (mg N g^{-1} soil)	0.33	15	0.89	0.57	0.56	**0.009**	0.19	**0.09**	0.37
0.05–0.25-mm WSA C:N ratio (g C g^{-1} N)	0.90	14	**< 0.001**	0.46	0.38	**< 0.001**	**< 0.001**	0.23	0.43

Note: Analyses do not include initial soil condition shown in figures.
[a]Mean-weight diameter; [b]water-stable aggregates.

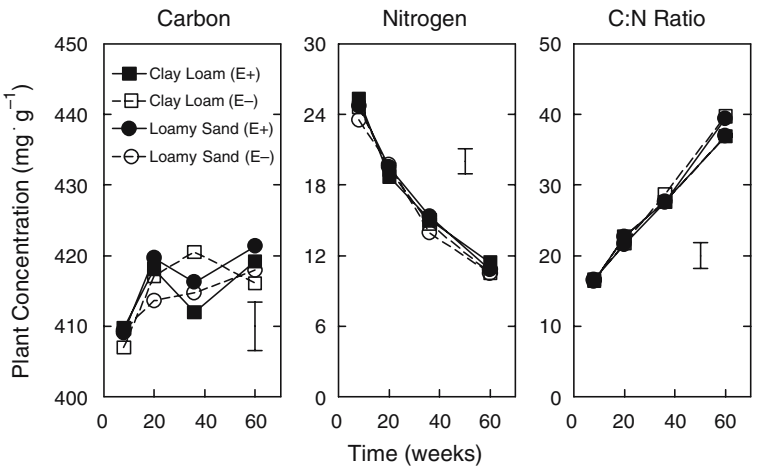

Figure 3. Above-ground plant concentration of C and N as affected by endophyte infection (E− is endophyte-free, E+ is endophyte-infected) in two soils harvested at different times during a 60-week growth period. * indicates difference between endophyte infection means within a soil type and sampling period at $P = 0.1$. Error bar denotes least significant difference among all means (sampling period, soil type, and endophyte infection) within a panel at $P = 0.1$.

and relatively lower in the loamy sand. Most of this interactive effect occurred early in the growth of tall fescue (Figure 4), as indicated by the significant soil type×endophyte×sampling period effect (Table 1). Due to the lower particulate organic N concentration under E+ than E− in the loamy sand, particulate C:N ratio was greater under E+ than E- in the loamy sand at 8 and 36 weeks of growth (Figure 4).

Mineralizable C and N were mostly affected by sampling period, but also by soil type (Table 1). Endophyte infection had no overall effect on mineralizable C and N, nor on microbial biomass C. Both mineralizable C and N increased with time, more so in the loamy sand than in the clay loam. Mineralizable C:N ratio decreased with time in both soils and endophyte infection levels, but was higher throughout the evaluation period in the clay loam than in the loamy sand. The only endophyte effect on mineralizable C or N occurred at 8 weeks with reduced C:N ratio under E+ than E− in the clay loam. This effect disappeared during the remainder of the study.

Soil aggregation characteristics were mostly affected by soil type, but also by sampling period (Table 1). Macroaggregate stability and mean-weight diameter stability were greater in the loamy sand than in the clay loam (Figure 5). The overall endophyte interaction with sampling period was not significant. However at 36 weeks, mean-weight diameter stability was greater under E+ than E− in both soils. At 8 weeks, mean-weight diameter stability and macroaggregate stability were lower under E+ than E− in the loamy sand.

Total C and N concentrations in water-stable aggregate fractions were always affected by sampling period and sometimes by soil type (Table 1). Increases in C and N concentration occurred with time similar to the results for whole soil. A greater portion of the increase in whole-soil organic C and N occurred in macroaggregates (>0.25 mm) than in microaggregates (<0.25 mm). An overall endophyte effect and its interaction with soil type or sampling period were not significant, except for N concentration in microaggregates (0.05–0.25 mm) and C:N ratio of small macroaggregates (0.25–1 mm). Nitrogen concentration of microaggregates became greater with time under E+ than E−, independent of soil type (Figure 6). The C:N ratio of small macroaggregates increased less with time under E+ than E− in the clay loam.

Initially, distribution of total organic C among aggregate soil fractions was $8 \pm 4\%$ in large macroaggregates (1–4.75 mm), $35 \pm 14\%$ in small macroaggregates (0.25–1 mm), $33 \pm 5\%$ in microaggregates (0.05–0.25 mm), and $24 \pm 4\%$ in free primary particles (<0.05 mm) when averaged across soil types and endophyte infection. At the end of the 60 weeks, this distribution shifted to a greater portion of the C in larger aggregate sizes, e.g., 28 ± 3, 40 ± 15, 22 ± 4, and $10 \pm 3\%$,

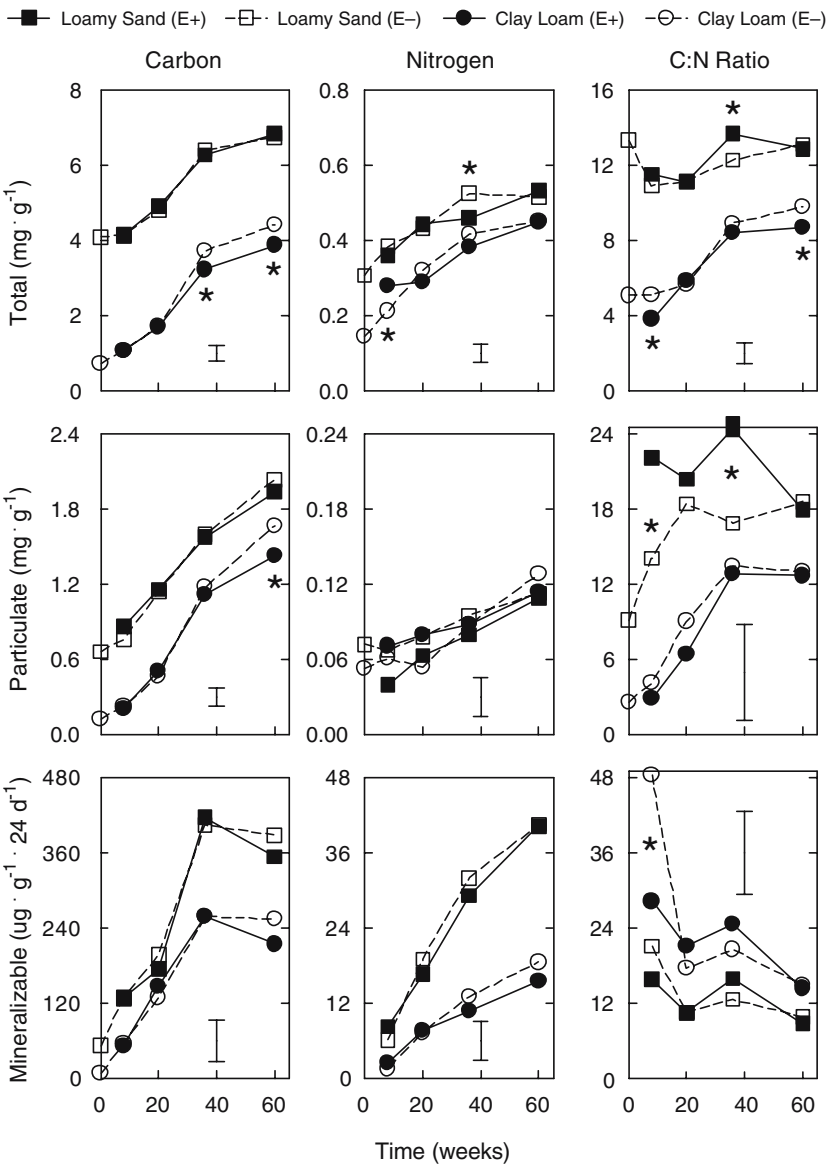

Figure 4. Total, particulate, and mineralizable C and N fractions in response to endophyte infection (E− is endophyte-free, E+ is endophyte-infected) in two soils harvested at different times during a 60-week growth period. * indicates difference between endophyte infection means within a soil type and sampling period at $P = 0.1$. Error bar denotes least significant difference among all means (sampling period, soil type, and endophyte infection) within a panel at $P = 0.1$.

respectively. Distribution of C among aggregate fractions was not influenced by endophyte infection nor its interactions with soil type or sampling period. A different distribution dynamic occurred for N, where initial and final percentages, respectively, were 6 ± 1 and $15 \pm 2\%$ in large macroaggregates, 40 ± 17 and $41 \pm 16\%$ in small macroaggregates, 43 ± 13 and $29 \pm 6\%$ in microaggregates, and 11 ± 10 and $15 \pm 11\%$ in free primary particles when averaged across soil type and endophyte infection. In addition, endophyte infection interacted significantly with soil type and sampling period to influence N distribution among all aggregate fractions < 1 mm. At the end of 60 weeks, the percentage of total N in small macroaggregates was greater under E+ (48%) than under E− (33%) in the clay loam, but lower under E+ (32%) than under E− (51%) in the loamy

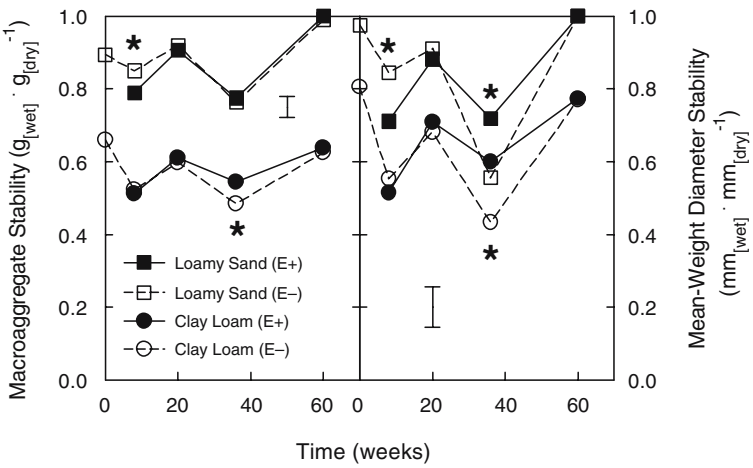

Figure 5. Stability of macroaggregates and mean-weight diameter in water in response to endophyte infection (E− is endophyte-free, E+ is endophyte-infected) in two soils harvested at different times during a 60-week growth period. * indicates difference between endophyte infection means within a soil type and sampling period at $P = 0.1$. Error bar denotes least significant difference among all means (sampling period, soil type, and endophyte infection) within a panel at $P = 0.1$.

sand. At the end of 60 weeks in microaggregates, N distribution was greater under E+ than E− in both the clay loam (32 and 13%, respectively) and in the loamy sand (40 and 31%, respectively). At the end of 60 weeks, the percentage of total N in primary particles was lower under E+ (5%) than under E− (37%) in the clay loam, but greater under E+ (15%) than under E− (2%) in the loamy sand.

Discussion

This short-term, semi-controlled mesocosm study confirmed that tall fescue dry matter production was positively affected by the presence of the fungal endophyte, *Neotyphodium*. This positive effect of endophyte infection on dry matter production has been demonstrated previously in several studies (Belesky et al., 1989; Clay, 1987). The positive effect of endophyte infection on productivity has been observed primarily with high fertility, as both Cheplick et al. (1989) and Arachevaleta et al. (1989) found increasingly greater dry matter production due to endophyte infection with increasing nutrient additions. The positive endophyte effect on plant dry matter production under high fertility conditions in this study was consistent with the findings of previous studies.

Endophyte effects on the various soil C and N fractions measured in this study were surprisingly few. It was hypothesized that relatively short-term exposure of soil to the tall fescue-endophyte association could possibly increase total organic C and N as was observed in long-term pastures (Franzluebbers et al., 1999; Franzluebbers and Stuedemann, 2005), primarily in response to altered soil microbial activity as was observed in short-term incubations (Franzluebbers and Hill, 2005; Omacini et al., 2004). Several reasons for the lack of endophyte effects on soil C and N fractions are possible. First, the conditions for altering soil C and N fractions due to the presence/absence of endophyte may not have been ideal in this study. Although forage was returned to the soil surface and allowed to decompose during this study, it may have been too little or too isolated from the soil processes that were being more dominated by large root contributions to soil C and N fractions. The fact that few endophyte effects occurred in the dominating presence of tall fescue roots suggests that roots may not play a major role in the endophyte effect on soil C and N fractions.

Whether ergot alkaloids, other alkaloids, or a combination of chemical compounds might be responsible for potentially altering soil C and N fractions is not known. It may be possible that ingestion of forage and subsequent excretion by grazing animals may be an important intermediate process to fully express the responsible mechanisms.

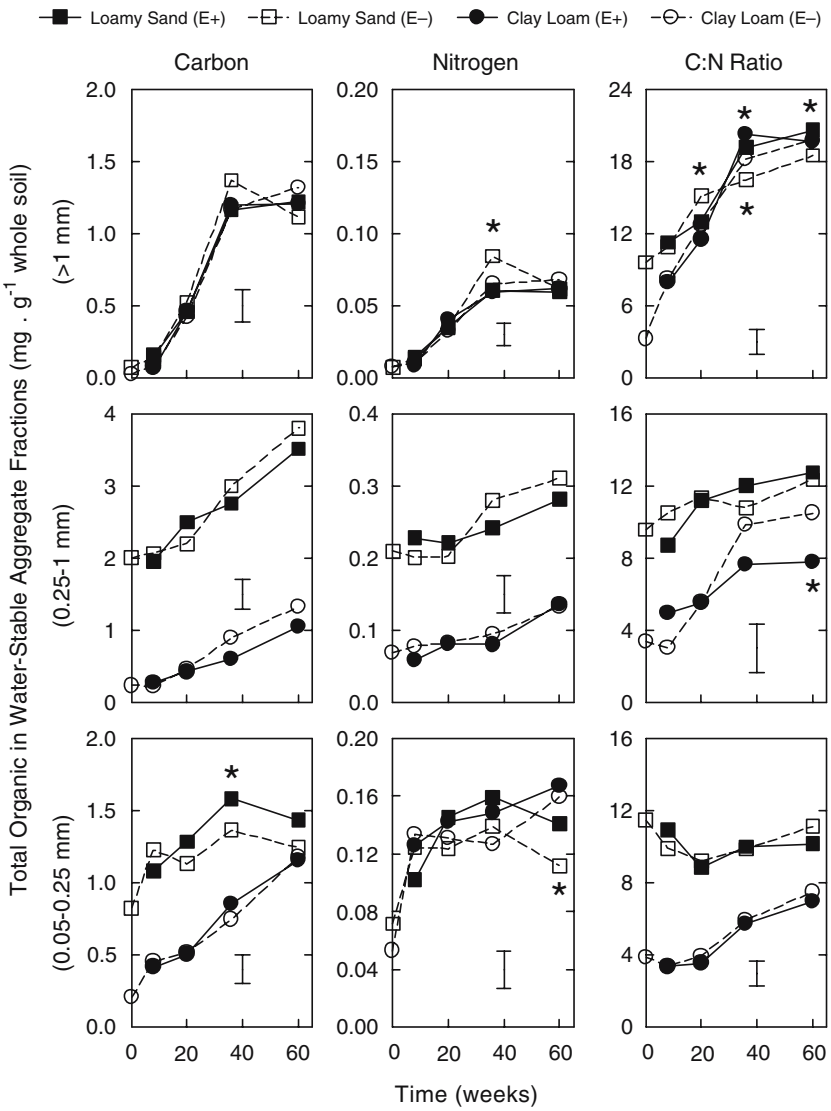

Figure 6. Total C and N in water-stable aggregate fractions in response to endophyte infection (E– is endophyte-free, E+ is endophyte-infected) in two soils harvested at different times during a 60-week growth period. * indicates difference between endophyte infection means within a soil type and sampling period at $P = 0.1$. Error bar denotes least significant difference among all means (sampling period, soil type, and endophyte infection) within a panel at $P = 0.1$.

It is possible that alterations in soil C and N fractions due to endophyte infection of tall fescue may have required >60 weeks to be detectable, especially in light of the rapid and dramatic overall changes in soil C and N fractions that occurred simply due to abundant plant growth. Detection of an endophyte effect should have been possible with the experimental setup employed, since the average rate of C accumulation observed in 8- to 15-year-old tall fescue pastures (Franzluebbers et al., 1999) would have been equivalent to 0.12 ± 0.08 mg C g^{-1} soil. The rate of C accumulation observed in this study was 3.0 ± 0.4 mg C g^{-1} soil $year^{-1}$, which could have overwhelmed the mechanistic endophyte effect on soil C and N cycling. The rate of total C and N accumulation observed in this study was equivalent to 4.17 ± 0.57 Mg C ha^{-1} $year^{-1}$ and 250 ± 59 kg N ha^{-1} $year^{-1}$, which would be in the high range of values reported even for grass management systems (Follett et al., 2001).

The fraction of total C and N that was particulate organic C and N increased during the course of this study as a result of abundant rhizodeposition. The fraction of total C as particulate organic C at initiation was 0.20 ± 0.01 and at the end of 60 weeks was 0.34 ± 0.05. The fraction of total N as particulate organic N increased from 0.19 ± 0.07 at initiation to only 0.23 ± 0.02 at the end of 60 weeks. Although particulate organic C and N contributed to the increase in total C and N with time, they accounted for only a small portion of the total change that occurred. Why the rate of particulate organic C accumulation was lower under E+ than E− is curious, since previous observation from long-term pastures indicated greater accumulation of particulate organic C with high than with low endophyte infection (Franzluebbers and Stuedemann, 2002). The interaction of soil type and sampling period with endophyte infection on particulate organic N was also a result that is not easily explained. Early accumulation of particulate organic N with E+ than E− in the clay loam was counteracted with lower accumulation with E+ than E− in the loamy sand. Lemons et al. (2005) found interactions between endophyte infection and soil invertebrate populations. Since our study was conducted outdoors, changes in soil invertebrate populations could have occurred during the course of the study and modified endophyte responses.

This study demonstrated the overwhelming influence of plant root production of this perennial, cool-season grass on the accumulation of active and slow fractions of soil C and N. Although aggregate stability indices did not change dramatically in this study, there was some fluctuation with time and endophyte infection suggesting that root activity and soil water content may have interacted with microbial dynamics responsible in cementing soil particles. Deposition of C and N occurred in all water-stable aggregate fractions, but was not consistent with observations in a 20-year-old pasture experiment, where high endophyte infection led to greater C and N in macroaggregates (>0.25 mm) and no difference in microaggregates (<0.25 mm) (Franzluebbers and Stuedemann, 2005). In contrast, this mesocosm study indicated that E+ increased C and N accumulation in microaggregates more so than E−. It is likely that soil C and N distribution in aggregates will be temporally dependent upon soil water and microbial activity fluctuations, as well as faunal perturbations that could shift resources from stable macroaggregate assemblages of microaggregates into free microaggregates (Six et al., 2002).

Conclusions

Endophyte-infected tall fescue resulted in greater above- and below-ground dry matter production than endophyte-free tall fescue. This greater plant productivity response to endophyte infection was not accompanied by significant and consistent changes in short-term soil C and N cycling among various active, slow, and aggregate-associated fractions. Large accumulation of soil C and N fractions occurred with time due to plant root-derived contributions, which could have overwhelmed any mechanistic endophyte effects on soil C and N fractions. This study suggests that greater plant productivity of endophyte-infected tall fescue is likely a contributing mechanism for eventual changes in total and active C and N fractions that have been observed in long-term pastures.

Note

[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.

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References

Arachevaleta M, Bacon C W, Hoveland C S and Radcliffe D E 1989 Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81, 83–90.

Beare M H, Cabrera M L, Hendrix P F and Coleman D C 1994 Aggregate-protected and unprotected organic matter pools in convetional- and no-tillage soils. Soil Sci. Soc. Am. J. 58, 787–795.

Belesky D P, Stringer W C and Hill N S 1989 Influence of endophyte and water regime upon tall fescue accessions. I. Growth characteristics. Ann. Bot. (London) 63, 495–503.

Bundy L G, Meisinger J J, 1994 Nitrogen availability indices. *In* Methods of Soil Analysis, Part 2. Eds. Weaver R W, Angle J S and Bottomley P S. pp. 951–984. Soil Sci. Soc. Am. Book Ser. 5, Soil Sci. Soc. Am. Madison, WI.

Cambardella C A and Elliott E T 1992 Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56, 777–783.

Cheplick G P, Clay K and Wray S 1989 Interactions between fungal endophyte infection and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. New Phytol. 111, 89–97.

Clay K 1987 Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. Oecologia 73, 358–362.

Clay K 1993 The ecology and evolution of endophytes. Agric. Ecosyst. Environ. 44, 39–64.

Clay K 1997 Consequences of endophyte-infected grasses on plant biodiversity. *In* Neotyphodium/Grass Interactions. Eds. C W Bacon and N S Hill. pp. 109–124. Plenum Press, New York.

Elliott E T 1986 Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.

Follett R F, Kimble J M, Lal R (Eds.) 2001 The Potential of U.S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. Lewis Publications, Boca Raton FL, 442 pp.

Franzluebbers A J and Hill N S 2005 Soil carbon, nitrogen, and ergot alkaloids with short- and long-term exposure to endophyte-infected and endophyte-free tall fescue. Soil Sci. Soc. Am. J. 69, 404–412.

Franzluebbers A J, Nazih N, Stuedemann J A, Fuhrmann J J, Schomberg H H and Hartel P G 1999 Soil carbon and nitrogen pools under low- and high-endophyte-infected tall fescue. Soil Sci. Soc. Am. J. 63, 1687–1694.

Franzluebbers A J and Stuedemann J A 2002 Particulate and non-particulate fractions of soil organic carbon under pastures in the Southern Piedmont USA. Environ. Pollut. 116, S53–S62.

Franzluebbers A J and Stuedemann J A 2005 Soil carbon and nitrogen pools in response to tall fescue endophyte infection, fertilization, and cultivar. Soil Sci. Soc. Am. J. 69, 396–403.

Hiatt EE and Hill NS 1999 Tall fescue endophyte detection: Tissue immunoblot test kit compared with microscopic analysis. Crop Sci. 39, 796–799.

Hoveland C S 1993 Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. Agric. Ecosyst. Environ. 44, 3–12.

Kemper W D and Koch E J 1966 Aggregate stability of soils from western United States and Canada. USDA Tech. Bull. 1355. U.S. Govt. Print. Off, Washington, DC.

Lemons A, Clay K and Rudgers J A 2005 Connecting plant-microbial interactions above and belowground: A fungal endophyte affects decomposition. Oecologia 145, 595–604.

Omacini M, Chaneton E J, Ghersa C M and Otero P 2004 Do foliar endophytes affect grass litter decomposition? A microcosm approach using *Lolium multiflorum*. Oikos 104, 581–590.

Paul E A, Clark F E, 1996 Soil Microbiology and Biochemistry. Academic Press, San Diego CA, 340 pp.

Six J, Conant R T, Paul E A and Paustian K 2002 Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155–176.

Stuedemann J A and Hoveland C S 1988 Fescue endophyte: history and impact on animal agriculture. J. Prod. Agric. 1, 39–44.

Tisdall J M and Oades J M 1982 Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.

Voroney R P and Paul E A 1984 Determination of kC and kN in situ for calibration of the chloroform fumigation-incubation method. Soil Biol. Biochem. 16, 9–14.

Wander M M 2004 Soil organic matter fractions and their relevance to soil function. *In* Soil Organic Matter in Sustainable Agriculture. Eds. F Magdoff and R R Weil. pp. 67–102. CRC Press, Boca Raton, FL.

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