Enzyme activity in a sinkhole undergoing forage renovation

Essam El-Naggar[A], **Mark Coyne[A]** and Tim Phillips[A]

[A]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA, Email emel222@uky.edu, mscoyn00@email.uky.edu, tphillip@uky.edu

Abstract
Endophyte-infected tall fescue is a dominant forage grass in the southeastern United States. Efforts are being made to find replacement forages for animal production, but knowledge about such renovation effects on soil biological properties is limited. We compared soil enzyme activity at two depths in three tall fescue cultivars differing in endophyte infection status. The tall fescue was planted in 160 m-long transects spanning a sinkhole, which represents a typical topographic feature in central Kentucky pastures. Soil dehydrogenase and β-glucosidase activity were significantly higher at 0-15 than 15-30 cm depth. At 0-15 cm depth both enzymes had significantly lower activity in the newly established grasses than in the undisturbed control. Soil dehydrogenase activity declined in the order: Undisturbed pasture (control) > KYFa9301 (endophyte-free) = KY 31 (endophyte infected and alkaloid-producing) > KYFa9301/A584 (endophyte-infected, alkaloid-free). β-glucosidase activity declined in the order: Undisturbed pasture (control) = KYFa9301 > KY 31 = KYFa9301/A584. No statistically significant correlation was found between enzyme activity and soil pH, TC, TN, clay content, or moisture content. The effects of forage transition were observable in the short term, and presence or absence of endophyte in tall fescue appears to affect soil biological activity.

Key Words
Soil dehydrogenase, β-glucosidase, tall fescue, Neotyphodium coenophialum, karst topography.

Introduction
Tall fescue (*Festuca arundinaceae* Schreb.) is a cool-season forage grass that dominates pastures in the southeastern United States. Kentucky 31 (KY 31) is a dominant cultivar in this region, but unfortunately suffers from infection by the endophyte *Neotyphodium coenophialum*, which produces various ergot alkaloids (*e.g.* lysergic acid, ergovaline) that are detrimental to animal growth and productivity (Siegel and Bush 1996). Much effort is currently devoted to replacing KY 31 with alternate forage species, including endophyte or alkaloid free tall fescue. Although there has been some research on the effects of such transition in terms of soil C (Franzluebbers *et al.*1999; Franzluebbers and Hill 2005; Franzluebbers and Stuedmann 2005) little is known about the immediate effects of transition on sensitive soil quality indicators reflecting C dynamics. In central Kentucky, potential effects of forage transition are exacerbated by the underlying karst topography. Numerous sinkholes that develop in this landscape provide rapid conduits for surface contaminants into shallow groundwater resources. Howell *et al.* (1995) have already demonstrated that fecal coliforms from grazing cattle in karst environments can rapidly percolate through soil into springs and streams. Transition to forages more palatable to grazing animals could therefore have unintended detrimental effects with respect to groundwater quality. The objective of this study was to determine if transition from native pasture to one of three tall fescue cultivars with or without endophyte and/or alkaloid production would influence enzyme indicators of soil quality related to C transformations.

Methods
Research site
The research was performed at the University of Kentucky Animal Research Center in Woodford Co., Kentucky, USA, approximately 18 km east of Lexington. The soil at this site is classified as a Maury silt loam (Typic hapludalf) with 6-12% slopes. An existing sinkhole in permanent pasture – a mixture of Kentucky bluegrass (*Poa pratensis* Linn.) and tall fescue – was treated with glyphosate herbicide (Roundup™) to remove the existing vegetation in Fall 2008, and again in Spring 2009. Three tall fescue cultivars were direct seeded into the killed sod in April 2009:

1. Control – Existing pasture
2. KY 31 – Endophyte infected and alkaloid-producing tall fescue
3. KYFa9301/A584 – Endophyte infected, non alkaloid-producing tall fescue
4. KYFa9301 – Endophyte- and alkaloid-free tall fescue.

The experiment design consisted of four nonreplicated treatments spanning the sinkhole. Each treatment was a strip 160 m-long by 3 m-wide separated by 2 m-buffers. In July 2009, after the new forages were well established, soils were collected at 10-m intervals along four transects representing the three forage treatments and an undisturbed control (Figure 1). Each location was sampled at two depths: 0-15 and 15-30 cm. Soils were manipulated to break up large aggregates, air dried, and stored at 4° C until use. To make treatment comparisons of enzyme activity paired difference *t*-tests assuming equal variance were performed for enzyme activity in each treatment at each sampling location with the null hypothesis of no treatment difference between treatments for the entire population of sample locations. For the purpose of statistical analysis the design was treated as a systematic distribution (Hurlbert 1984).

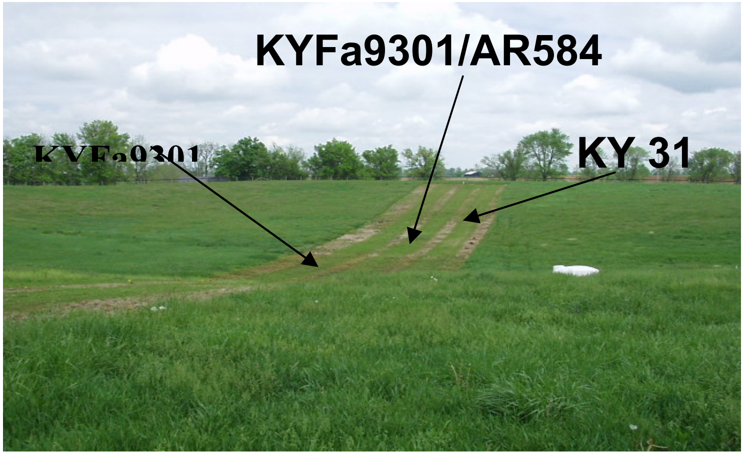

Figure 1. Distribution of forage treatments in the sinkhole site at the University of Kentucky Animal Research Center in Woodford Co.

Soil enzyme activity

Soil dehydrogenase was assessed colorimetrically in triplicate by the method of Tabatabai (1996), which measures the production of TPF (Triphenyltetrazolium formazon) from the reduction of TTC (Triphenyltetrazolium chloride) in samples incubated at 37° C for 24 h. β-glucosidase activity was measured colorimetrically in triplicate by the production of para nitrophenol (PNP) from the hydrolysis of para nitrophenol- β-D-glucopyranoside in samples incubated 1 h at 37° C (Tabatabai 1996). Both sets of soil enzyme activity utilized reagent blanks to correct for background absorbance.

Results

The experiment design and the varied topography precluded a simple comparison of treatment means for each enzyme. To minimize the potential for pseudoreplication we compared differences among treatments at each sampling location. This approach does not preclude a systematic bias across treatment locations at each sampling location, but spatial analysis indicated that within the region encompassed by each sampling location soil properties (pH, total C, total N, clay content) were random and therefore enzyme differences between treatments were ascribed to treatment effects (El-Naggar *et al* 2010). Soil Enzyme Activity Soil dehydrogenase activity was significantly higher ($p < 0.05$) at 0-15 than 15-30 cm in all treatments (Figure 2). Likewise, the dehydrogenase activity in the control was significantly higher ($p < 0.05$) in the control compared to all other treatments. Cultivar KYFa9301/A584, which is infected by a novel non-alkaloid-producing endophyte, had significantly lower soil dehydrogenase activity than the other two treatments.

β-glucosidase activity was significantly higher ($p< 0.01$) at 0-15 cm than 15-30 cm in all treatments. Activity in the control ranged from 147 to 228 µg PNP g^{-1} h^{-1} at 0-15 cm (Figure 3), which was significantly ($p< 0.05$) higher than fescue treatments containing endophytes for the same depth. There were also significant differences between the fescue treatments at 0-15 cm depth with the general trend for activity being: Undisturbed pasture (control) = KYFa9301 > KY 31 = KYFa9301/A584 (Figure 4).

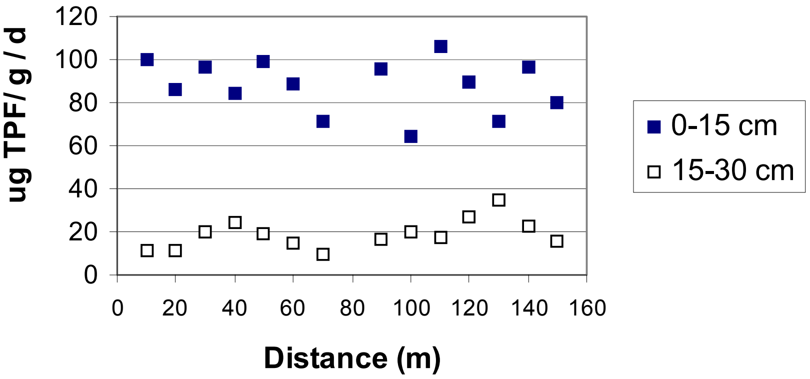

Figure 2. Soil dehydrogenase activity in the undisturbed control at two depths. All other treatments showed the same result, and are omitted for simplicity. The center of the sinkhole appears at approximately 80 m distance.

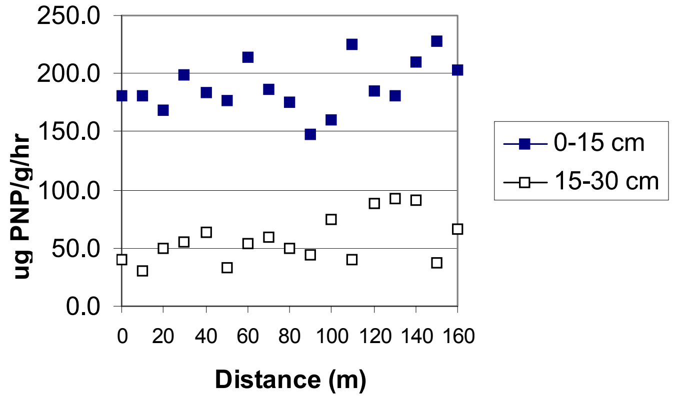

Figure 3. β-glucosidase activity in the undisturbed control sample at two soil depths. All other treatments showed the same result, and are omitted for simplicity. The center of the sinkhole appears at approximately 80 m distance.

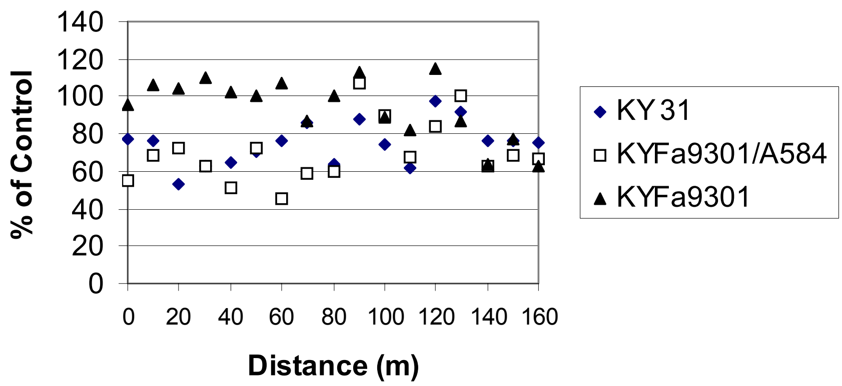

Figure 4. β -glucosidase activity in fescue treatments at 0-15 cm depth relative to the undisturbed control at each position.

Conclusion

Four months after pasture renovation and replacement of the existing sod with alternative fescue cultivars there were clear differences in the level of dehydrogenase and β-glucosidase activity. In each case the transition resulted in decreased activity, which indicates that procedures for pasture renovation that involve killing the existing sod with herbicide before reseeding can have an adverse effect on soil biological activity. There was some evidence that the choice of replacement fescue cultivars had an influence on the subsequent soil enzyme activity. KYFa9301/A584, which is a KY 31 derivative containing a novel endophyte that produces no alkaloid, typically had the lowest soil enzyme activity of any of the cultivars tested. Despite having a symmetrical appearance, the sinkhole was asymmetric with respect to enzyme activity. There was evidence for spatial organization of the enzyme data (El –Naggar *et al* 2010). Most likely, this was because the south-facing slope for the distance 80-160m was both warmer and shallower than the north-facing slope

Acknowledgements
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