

# Spatial variability of soil enzymes in a sinkhole undergoing forage transition

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## Abstract

Tall fescue is a dominant forage grass in the southeastern United States. The dominant fescue cultivar, KY 31, is infected with an alkaloid-producing endophyte, and research is evaluating new forage species and endophyte-free cultivars as pasture replacements. Spatial distribution of soil enzymes affecting soil processes in transitional forage systems is not well-studied. We evaluated the spatial variability of soil enzymes involved in C metabolism at a sinkhole site in central Kentucky, USA. Dehydrogenase and  $\beta$ -glucosidase were selected because they reflect soil microbiological activity status and play a key role in bioprocesses involving C cycling. There was evidence of spatial variability for enzyme activity across the sinkhole. Dehydrogenase activity showed moderate spatial structure at 15-30 cm depth compared to strong spatial structure over a 100 m range under endophyte-infected KYFA9301/A584 and KY31. There was moderate spatial correlation over a 60 m range under endophyte-free KYFA9301, and a complete nugget effect under the undisturbed control. Spatial analysis of  $\beta$ -glucosidase activity indicated a weak to moderate spatial structure over a 40 to 100 m range under different forage species, except for a pure nugget at 15-30 cm depth under KY31 and KYFA9301/A584. Spatial cross correlation analysis showed that both enzymes are spatially correlated for two soil depths over a range from 10 to 30 m. Cross semivariance analysis showed spatial independence between soil enzyme activity and TN, soil pH, and clay content.

## Key Words

Enzyme activity, dehydrogenase,  $\beta$ -glucosidase, geostatistics, karst, nugget effect, cross variograms.

## 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 suffers from infection by the endophyte *Neotyphodium coenophialum*, which produces ergot alkaloids that are detrimental to animal growth and productivity. There has been research on the effects of endophyte-infected fescue on soil C dynamics (Franzluebbbers and Stuedmann 2005) but relatively little is known about spatial variability of soil processes in fescue-dominated pastures, particularly those pastures undergoing transition to remove endophyte-infected species (Franzluebbbers *et al.* 1999).

In central Kentucky, potential effects of forage transition are exacerbated by the underlying karst topography. Karst topography creates soil environments dominated by multiple sinkholes that differ in width and depth. These sinkholes provide rapid conduits for surface contaminants into shallow groundwater resources. Spatial variability of soil properties and processes is likely to be influenced within very short distances in this landscape. With respect to soil enzymatic properties, little is known about their spatial variability in such environments. Characterizing the effect of changing soil vegetation cover on spatially variable patterns of soil biological properties such as soil enzyme activity will help us understand the process of soil organic mass transformation and its impacts on other soil chemical and physical properties. Eventually, these types of studies will help us predict the environmental response of soil ecosystems to this change. The objectives of this study were to test the hypotheses that soil enzyme properties had spatial structure in a sinkhole environment and that this spatial structure was affected by transition from the existing forage.

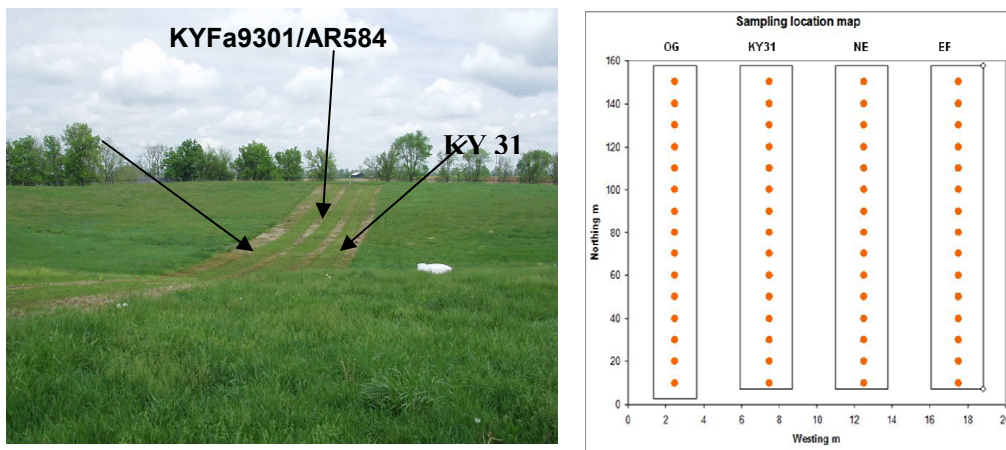
## Methods

### 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. Along with undisturbed sod, three tall fescue cultivars were direct seeded into the killed sod in April 2009 in individual

strips of 160 x 3/m<sup>2</sup> spanning the center of the sinkhole (Figure 1) to give four treatments: 1). Control (OG) – Existing pasture; 2). KY 31 – Endophyte infected and alkaloid-producing tall fescue; 3). KYFa9301/A584 (NE) – Endophyte-infected, non alkaloid-producing tall fescue; 4). KYFa9301 (EF) – Endophyte- and alkaloid-free tall fescue.

In July 2009, after the new forages were well established, soils were collected at 10-m intervals along four transects, each transect representing one of the three forage treatments and the 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 enzyme analysis for soil dehydrogenase and β-glucosidase.



**Figure 1. Distribution of forage treatments, transect locations, and sampling positions in the sinkhole research site at the University of Kentucky Animal Research Center in Woodford Co.**

### *Spatial Analysis*

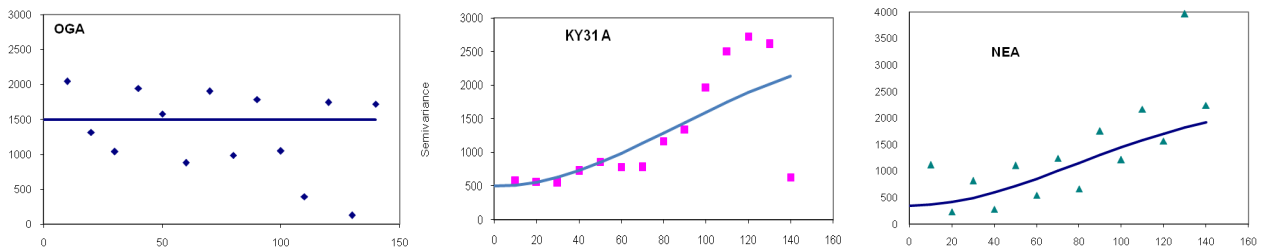
Spatial structure and spatial variations of soil enzymes activities were described using omni-direction semi-variograms assuming horizontal isotropy (Goovaerts 1997; Deutsch and Journel 1998; Chiles and Delfiner 1999). Visual and statistical approaches were used for variogram modeling (Webster and Oliver 2001). Spherical and Gaussian models were identified for the best spatial model that fit soil enzyme variograms in different forage species based on the residual sum of squares (RSS) analysis. The ratio between the nugget variance and total variance was used to evaluate the spatial dependence and spatial structure in the different forage species (Cambardella *et al.* 1994). Nugget values of the two soil enzymes in the different forage species represent the undetectable experimental error, field variation associated with the minimum sampling distance, and inherent variability. Cross variogram analysis, Yates and Warrick (2002) and Nielsen and Wendroth (2003) was applied to test the cross continuity between the two soil enzymes.

## **Results**

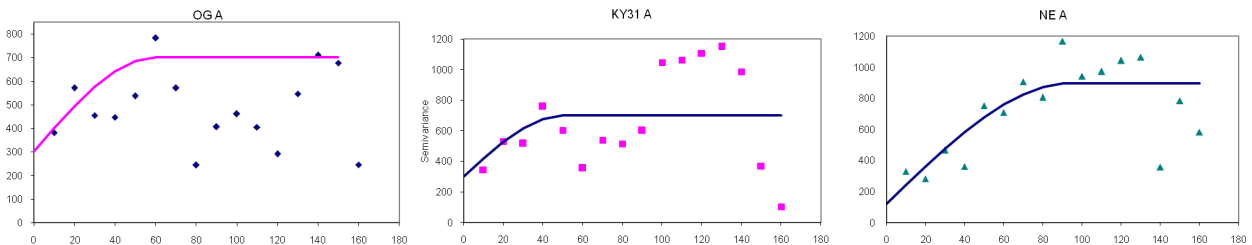
### *Spatial Variability*

Because we collected samples in a systematic way with a fixed separation distance – a non randomized design – spatial dependency needed to be taken into account by using spatial analysis methods described below. Traditional statistics assumes that all samples are collected using a randomized sampling design. Our results show that assumptions of random spatial distribution of enzyme activities are not valid. Sampling separation distance was valid to capture the spatial structure, except under the OG treatment, where a shorter separation distance between samples needed to be applied. There is a spatial dependency between the two enzymes and between enzymes and the TC content under different all cultivars except for the original forage.

Sample comparison of the spatial semi-variance as a function of separation distance for the two soil enzymes under different forage species are presented in Figures 2 and 3. Spherical and Gaussian models were used to fit the semivariance as a function of separation distance. Strong spatial structure for dehydrogenase activity at 0-15 cm was observed under KY31 and NE compared with a moderate spatial structure under EF. Dehydrogenase activity under the original pasture showed no spatial structure, and the semivariance was modeled as a pure nugget, which indicated that a longer spatial domain or shorter sampling distance needed to be applied. For 15-30 cm, a strong spatial structure over an 80 m range was observed under KY31 and EF compared with a moderate to weak spatial structure over a shorter correlation range (30-40 m) under OG and



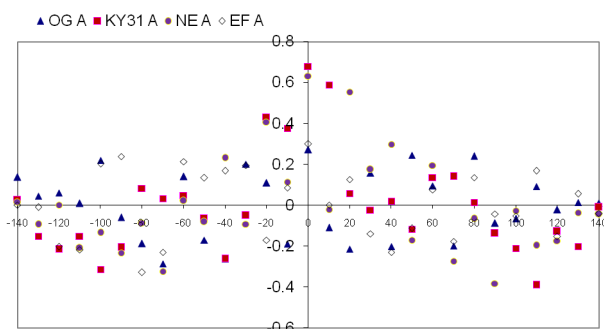
**Figure 2. Dehydrogenase activity semivariograms for 0-15 cm under different forage species.**



**Figure 3.  $\beta$ -glucosidase semivariograms for 0-15 cm under different forage species.**

NE. The dehydrogenase activity semivariograms under KY31 for the two soil depths and for the 15-30 cm depth under the EF show a trend effect starting after an 80 to 100 m range. This trend effect can be explained as a direct result of higher organic matter content in the northern part of the transect, compared with the first 100 m.

Semivariograms of  $\beta$ -glucosidase activity at 0-15 cm showed strong to moderate spatial structure over a 40 to 60 m correlation range in KY31 and NE compared with somewhat weak structure under OG and EF.  $\beta$ -glucosidase activity at 15-30 cm showed no spatial structure under KY31 and NE compared with somewhat moderate to weak spatial structure under EF and the control, respectively. Semivariograms of  $\beta$ -glucosidase showed a second increase in the semivariance starting between 80 to a 100 m of the transect. This trend is clear under the KY31 and NE at 0-15 cm depth and in OG and KY31 at 15-30 cm depth. This trend is also attributed to the higher content of soil organic matter content in the northern part of the transect. Cross variogram analysis between soil enzymes and selected soil properties (*i.e.* TC, TN, soil pH, clay content) showed a positive and strong cross correlation only between TC and the activity of the soil enzymes under OG, KY31, and NE in the two sampling depths. The cross correlation between TC and enzyme activity ranged between 50 to 120 m, which indicates strong spatial dependence between soil enzyme activities and TC. The cross correlation function (CCF) used to visualize the spatial dependence between the enzymes under different forage species is shown in Figure 4.



**Figure 4. Cross-correlation function between the soil enzymes.**

The CCF at lag equal zero is the same as the classical correlation coefficient between the two variables. Unlike classical statistics, the CCF analysis with distance gives insights about the spatial covariance structure between the two variables. There was spatial dependence between the soil enzymes in different forage species in a range of 10 to 30 m. A positive and significant cross correlation between the enzymes was observed in KY31 and NE compared to positive but non significant correlation in OG and EF at 0-15 cm. The results also showed positive and significant cross correlation between the two enzymes at 15-30 cm, but for a shorter range. This information can help in the spatial interpolation and estimation of the magnitude of a variable by knowing the magnitude of the neighborhood.

## Conclusion

Despite a symmetrical appearance, the sinkhole was asymmetric with respect to enzyme activity. Soil dehydrogenase and  $\beta$ -glucosidase exhibited strong to moderate spatial structure for the tested soil depths in different forage species. The degree of spatial structure within the tested domain was clearly affected by the forage species and inherited soil variability factors. Spatial patterns of enzyme activity were affected by forage species, and were associated with inherent soil organic carbon rather than total nitrogen content, clay content, or soil pH. These findings have important implications for understanding the process of soil organic mass transformation and for selecting an appropriate methodology of soil sampling for such studies. The factors responsible for the heterogeneity are only partially clear, and the source of the enzyme activity variation within horizontal and vertical domains remains to be clarified. For more accurate analytical results, such as more detailed spatial distribution patterns, it is necessary that a greater sampling density in both horizontal and vertical directions should be used.

## Acknowledgements

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