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# EFFECT OF TALL FESCUE ENDOPHYTE STRAINS AND NITROGEN FERTILIZATION ON SOIL AGGREGATE STABILITY

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## Abstract

Recently, it has been reported that the infection of tall fescue with *Neotyphodium* spp. endophytes may induce changes in host grass physiology, especially in root morphology that could affect soil aggregate stability. Therefore, the effects of N management under stockpiled "Jesup" tall fescue (*Schedonorus phoenix*) infected with and without different endophytes (*Neotyphodium coenophialum*) on short-term response of aggregate stability in a Marietta loam soil (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) was investigated. The experimental design was split-split block replicated four times. Endophyte strains in tall fescue evaluated were: endophyte-infected wild type (E+), novel endophyte infected (E502, E514, E542, and E584) and endophyte free (E-). Nitrogen treatment consisted of a control and two N sources [urea and ammonium nitrate ($NH_4NO_3$)] applied at two different rates (56 or 112 kg $ha^{-1}$ of actual N) applied in mid-September and mid-October. Aggregate stability was measured as water stable macro-aggregate (>250 µm) and micro-aggregate (125-250 µm) using a wet sieving apparatus. Endophytes in tall fescue did not affect macro-aggregates, but did affect micro-aggregates. Stable micro-aggregates decreased in following order E584 > E502 > E+ > E514 > E- > E542. The application of nitrogen fertilizer in September increased macro-aggregates by 4% and decreased micro-aggregates in the soil by 8% when applied as ammonium nitrate in comparison to urea. In contrast, application of N fertilizer in October increased micro-aggregates by 5% applied as ammonium nitrate in comparison to urea, but had no effect on macro-aggregates. These results indicate that the direction and magnitude of the change in stable aggregates in a soil could be influenced by the effect of management, climatic and biological factors on the wetting and drying cycle in the soil. Future studies are needed to determine if the effect of N fertilizer on aggregation could be attributed to the effect of the biomass yield on the soil moisture content at rewetting.

Keywords: aggregate stability, endophyte, nitrogen fertilizer, tall fescue, stockpiling

## INTRODUCTION

Tall fescue (*Festuca arundinaceae* Schreb.) is an economically important cool-season forage in Mississippi occupying an estimated 700,000 hectares (Lemus and Weirich, 2009). It is preferred to many other cool-season forages for stockpiling because it produces substantial growth in autumn retains its nutritive value longer in the winter and latedefoliation has little effect on the regrowth in the following season (Kallenbach et al., 2003; Burns et al., 2006). Stockpiling tall fescue not only extends the grazing season, but can also provide soil cover and increase soil aggregate stability to control runoff and erosion.

Aggregate stability imparts the potential of soil to resist the destructive actions of wetting, raindrop impact, and cultivation (Wuddivira and Camps-Roach, 2007). Levy and Miller (1997) found a direct, positive relationship between aggregate stability and infiltration, runoff and erosion in southern coastal plain soils. Since aggregate structure has limited resistance to weathering the aggregated soils needs to support a biological canopy to protect the soil. Well protected soils should also have sufficiently granulated and stable aggregates to allow the water to penetrate rapidly.

To produce high quality stockpiled tall fescue, a pasture should be grazed short (~8 cm) or clipped and fertilized with N from mid-August to mid-September. There have been varied results and recommendations on when N should be applied, but most research show higher yields with earlier fertilization dates (Gerish et al., 1994; Collins and Balasko, 1981). Also, the yield of stockpiled fescue is influenced by the choice of N source (Teutsch et al., 2005). Teutsch et al. (2005) compared the different N-sources for stockpiling tall fescue and found ammonium nitrate compared to urea to be the best source of N for stockpiling tall fescue. These results suggest higher stockpiled yields can be achieved with an early application of ammonium nitrate.

Most tall fescue plants are infected with the endophytic fungus *Neotyphodium coenophialum*. Endophyte-infected tall fescue produces ergot alkaloids that are not only toxic to livestock, but result in reduced insect feeding and damage as in comparison to endophyte-free tall fescue (Malinowski and Belesky, 2000). Although the endophyte can be harmful to the livestock, it is actually helpful to the tall fescue plant. Researchers have discovered entophytes that do not produce the ergot alkaloid and are not toxic to animals. These endophytes are commonly referred to as non-toxic or “novel”.

Several investigators have compared yield of novel endophyte, endophyte infected (E+) and endophyte free (E-) tall fescue. Burns et al. (2006) investigated stockpiling of “Jesup” tall fescue without (E-) and with novel and wild-type fungal endophyte (E+). Research showed that endophyte status did not influence total forage biomass or forage consumed by grazing animals. On the other hand, the effect of endophyte status on nutritive value, ignoring ergovaline concentration and effects, was greatest in the wild type. Other research has reported that endophyte infection of perennial ryegrass and meadow tall fescue did not affect either dry matter yield or plant N uptake (Lewis et al., 1996; Ravel et al., 1997). Endophyte effects on dry matter production of tall fescue and perennial ryegrass seedlings grown under optimal nutrient supply resulted in greater tiller number and above-ground biomass of endophyte-infected plants than non-infected plants for both species (Clay, 1987). Bouton et al. (2002) and Kay et al. (2001) also reported higher dry matter yields for endophyte infected “Jesup” and Kentucky 31(K-31) tall fescues than for endophyte free “Jesup” and Ky-31. These inconsistent results suggest a specific interaction of endophyte and host grass associations on tall fescue production which might be also influenced by environment and management.

Studies conducted on the formation and stabilization of aggregates showed that roots and mycorrhizal fungal hyphae are the major binding agents responsible for the formation of macro-aggregates (>250µm) in soils (Tisdall, 1980; Tisdall and Oades, 1982; Haynes and Francis, 1993; Haynes and Beare, 1997). Malinowski and Belesky (2000) reported that the infection of tall fescue with *Neotyphodium* spp. endophytes induces changes in host grass physiology, especially in root morphology. In their earlier work, Malinowski et al. (1999) found smaller root diameter and longer root hairs in hydroponically grown E+ in comparison to E- genotypes of tall fescue. Endophyte in grasses has also been shown to reduce mycorrhizal colonization of the host roots as well as spore densities in the soil (Chu-Chou et al., 1992; Guo et al., 1992; Mueller, 2003; Keenan et al., 2008).

Previous studies have reported inconsistent results on the effects of N fertilization on aggregate stability. Some studies have reported that the aggregate stability decreases with increased N fertilization (Latif et al., 1992; Foster et al., 2009); however, Haynes and Beare (1996) reported that the aggregate stability increases with N fertilization with an increase in organic matter and concurrent increases in crop yield and crop residue. Furthermore, Haynes and Naidu (1998) reported that application of $NH_4^+$ containing or forming fertilizers can have an adverse effect on soil aggregation under low temperature, low moisture and low pH, the same conditions which inhibit nitrification. An understanding of the effects of N fertilizer application on aggregate stability under stockpiled tall fescue is critical for the development of science based soil and water conservation strategies. The objective of this research was to determine the effects of N fertilizer treatments and date of application on short-term response of aggregate stability under stockpiled novel tall fescue infected with and without different endophytes.

## MATERIALS AND METHODS

Tall fescue plots were established in the fall of 2004 at the H.H. Leveck Animal Research Center, Mississippi State University on a Marietta loam soil (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) to study the short-term response of water stable aggregates of soil planted with "Jesup" tall fescue infected with different endophyte strains or no endophyte under different N managements. The endophyte strains were wild type (E+), novel endophytes (E502, E514, E542, and E584) and plants without endophyte (E-). Percent endophyte infection four years after establishment was 90, 83, 63, 55, 40 and 23% for E+, E584, E514, E542, E502 and E- respectively (Lemus, 2009). Nitrogen was applied at two different dates in the fall of 2008 and 2009 (September 15 and October 15). Three nitrogen rates (0, 56 and 112 kg N $ha^{-1}$) were applied using two nitrogen sources (urea and ammonium nitrate) at each application date. The experimental design was a Split-Split Block replicated four times. The whole plots were the fescue lines and the subplots were the application dates (September and October) and the sub-sub plots were the five nitrogen fertilizer treatments. The whole plot was 15 m x 5 m and each sub-plot was 3 m x 2 m with a 4 m alley between to minimize fertilizer runoff from one plot to another. The plots were harvested at the end of each growing season.

The soil was sampled on 1 April 2008 for soil texture, nutrient analysis and aggregate stability and on 18 March 2009 for aggregate stability. Composite samples (3 cores per plot to 15-cm depth) were taken from each plot, dried and sieved to pass 2 mm, to determine macro-aggregate stability (> 250 µm) and micro-aggregate stability (125-250µm). Composite samples (6 cores to 15-cm depth in each block) were taken from each block to determine soil texture.

For analysis of soil nutrient status, a composite sample of 20 random samples was taken throughout the plots. Soil was air-dried, sieved (2 mm), and a sub-sample analyzed for Mehlich III phosphorus and other chemical fertility parameter (Table 1). Phosphorus, K, Ca, and Mg were determined via emission spectroscopy on a Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma (ICP) Radial Spectrometer (Thermo Instrument Systems, Inc., Waltham, MA). Soil pH was determined on 1:2 soil:water slurries with a pH meter and glass electrode. The soil texture was determined by the method proposed by Olmstead et al. (1930).

**Water Stable Aggregates Determination**

Percentage water stable aggregates was determined based on the principle that unstable aggregates will break down more easily than stable aggregates when immersed in water using an Eijkelkamp Wet-Sieving apparatus after the method of Wuddivira and Camps-Roach (2007). A sub-sample of aggregates was air-dried for 48 hr. The aggregate stability of the 1-2 mm size fraction was determined by wet sieving. A single sieve apparatus with a stroke of 1.3 cm and a frequency of 34 cycle minute$^{-1}$ was used.

Four grams of air-dried aggregates on sieves were immersed in de-ionized water in cans (45 mm x 6.0 mm diameter) for 5 minutes and then the sieving was initiated for 3 minutes. The unstable aggregates pass through the sieve and were collected in a water filled can. After this fixed time (3 min), the cans were removed and replaced by cans with solution of 2 g NaOH per liter of distilled water. Aggregates remaining on the sieve were then sieved for 5 minutes in the NaOH solution, after which a rubber tipped rod, was rubbed across the screen. The sieving finished when all material passed through the screen except sand grains and plant roots. The pair of cans was placed in an oven at 110 °C for 24 hrs. Aggregate stability was calculated as the mass of stable aggregates (Ms) divided by the total aggregate (stable + unstable (Mu)) mass, and expressed as the percentage of water stable aggregates (WSA) (sand-free basis) (Wuddivira and Camps-Roach, 2007).

$$WSA = [(Ms/ (Ms + Mu)] \times 100 \qquad (1)$$

Aggregate stability was measured on sieve size 250 µm and on sieve of 125 µm. The percentage of stable micro-aggregates (125-250 µm) was determined by subtracting the amount of measured macro-aggregates (> 250 µm) from micro-aggregates (>125 µm) in each pot.

$$WSA_{(125\text{-}250\mu m)} = WSA_{(125)} - WSA_{(250)} \qquad (2)$$

Where $WSA_{(125)}$ is the amount of water stable aggregates measured on the 125 µm sieve size and $WSA_{(250)}$ is the amount of stable aggregates measured on the 250 µm sieve size.

**Statistical Analysis**

The PROC GLIMMIX procedure of Statistical Analysis System 9.2 (SAS Institute, 2009) was used to determine significance ($P \leq 0.05$) of sources of variation. Correlation analysis was performed with PROC CORR procedure. Mean separations were executed with Fisher’s protected least significant difference (LSD) at probability level $P < 0.05$.

## RESULTS AND DISCUSSION

In all treatments, the soil had more than 50 % of their weight in stable aggregates (> 125 µm) with about equal amounts of macro-aggregates (> 250 µm) and micro-aggregate (125-250 µm). The two factor interaction effect of date of application x nitrogen fertilizer was significant for macro-aggregates (> 250 µm) and micro-aggregates and the main effect of endophytes was significant for micro-aggregates (125-250µm) (Table 2).

Endophytes in tall fescue did not affect macro-aggregates, but did affect micro-aggregates (Table 2). Stable micro-aggregates decreased in following order: E584 > E502 > E+ > E514 > E- > E542. The result showed E584 left the soil with significantly more (2%) stable micro-aggregates than E542, E- and E514 (Fig. 1). The differences in micro-aggregates among the endophytes may be due to the effect of the endophyte on mycorrhizal colonization of roots and on root morphology (Malinowski and Belesky, 2000). The effect of endophytes in tall fescue on mycorrhizal colonization was confirmed by Keenan and Rudgers (2008). Keenan and Rudgers (2008) studied the asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungi endophytes. Their results showed a 50% reduction in mycorrhizal colonization in endophyte infected tall fescue. They concluded that endophyte density could alter the nutritional requirements of the host plant, thereby indirectly affecting mycorrhizal colonization. Mycorrhizal colonization or root characteristics such as root mass and root length were not measured in this study, but results show strong evidence that the endophyte in tall fescue may affect soil aggregate stability. Clearly, this difference of 2% may only be statistical and not biologically practical. However, more studies on practical implication of soils with varied aggregate stability levels are needed to determine relevance.

Statistical analysis found a significant interaction of date of application x nitrogen fertilizer on aggregate stability (Table 2) which was attributed to the N source. The result showed that application of nitrogen fertilizer in September increased macro-aggregates by 4% and decreased micro-aggregates in the soil by 8% when applied as ammonium nitrate in comparison to urea. In contrast, application of N fertilizer in October increased micro-aggregates by 5% applied as ammonium nitrate in comparison to urea, but had no effect on macro-aggregates. The biological significance of these differences is unknown. Therefore, without further studies relating these changes in soil aggregate stability on soil hydrological properties such as infiltration and runoff this statistical significance may be of little practical importance.

Soil aggregation is a complex soil process that is influenced by internal factors (soil texture, nutrient composition and organic matter level) and external factors (climate, plant species and management). Therefore, it may be difficult to identify a specific reason for the effect of N on macro-aggregates without measurements of other factors such as soil moisture, soil N concentration, root mass, age of the pasture, microbial population, and soil type.. Haynes and Naidu (1998) reported N fertilizer to directly affect macro-aggregates. They pointed out that when the monovalent $NH_4^+$ ion accumulates in soils in large amounts from applications of $NH_4^+$ containing or forming fertilizers it can become a dominant exchangeable cation and, like $Na^+$, it can have an adverse effect on soil aggregation. However, this is uncommon in most application of $NH_4^+$, particularly in the field, because too small a proportion of the soil volume is affected for too short a time (Haynes and

Naidu, 1998). Likewise, Latif et al. (1992) and Biederbeck et al. (1996) plot studies also reported a decrease in $WSA_{250}$ with high rates of N fertilizer as a result of the effect of N fertilizer on plant growth and plant environment. The decrease in $WSA_{250}$ in Biederbeck et al. (1996) study could be attributed to the low pH of the soil used, as low pH environment provides a favorable condition for accumulation of $NH_4^+$ (Haynes,1986). Latif et al., (1992) found that N fertilizer decreased $WSA_{250}$ under maize plots intercropped with legumes and increased WSA under maize only plots. The decrease in $WSA_{250}$ in Latif et al., (1992) study was attributed to the effect of N on root exudates which are known to affect aggregation. It is clear, that N fertilizer can influence $WSA_{250}$ directly through accumulation of $NH_4^+$, or indirectly through increase in biomass and microbial community.

We speculate that the increase in stable macro-aggregates from the application of ammonium nitrate in comparison to the application of urea in this study could be due to the indirect effect of the $NH_4^+$ on plant growth and soil environment. Soil moisture content was not measured throughout the growing the season in the study. Plant growth and warmer temperatures are known to influence the soil moisture content. Generally, moist conditions due to frequent rainfall events and cooler temperatures dominate the late fall and winter seasons in north Mississippi. Warmer temperatures in September in comparison to October are likely to result in more biomass yield with N fertilizer and affecting soil moisture content. The biomass yield showed ammonium nitrate to produce greater than 1700 $kgha^{-1}$ more in comparison to urea (Lemus, 2010). Dexter (1991) and Tisdall (1996) reported that as a soil dries, particles of clay, organic colloids and salts are deposited at points of contact, strengthening bonds between larger particles increasing soil aggregation. Rewetting a dry soil can cause air pressure to build up inside the aggregates resulting in breaking of the aggregates (Singer et al., 1992). Denef et al. (2002) reported that rewetting a moist soil tends to result in less breaking of the aggregates in comparison to rewetting a dry soil. The amount of stable macro-aggregates in a moist soil may increase following rewetting (Dexter, 1991; Denef et al., 2002). Our result indicates that the direction and magnitude of the change in stable macro-aggregates may be dependent on the soil moisture content at rewetting. Further studies are needed to evaluate the mechanism by which N fertilizer affect soil macro-aggregate stability.

The effect of N on micro-aggregates were in contrast to the findings of Tisdall and Oades (1980) that the more stable micro-aggregates (20 - 250 µm) are generally not affected by management. A scarcity of studies on the effect of N on aggregation prevents comparison with other findings.

Macro-aggregates were not correlated ($r = 0.15$) with the dry matter yield. This may be due to the similar effects found for the endopytes in tall fescue on macro-aggregates and dry matter yield. In contrast, micro-aggregates were negatively correlated ($r = -0.56$), but was not significant ($P = 0.2494$). Macro-aggregates are from the binding of micro-aggregates (Tisdall and Oades, 1982). The direction of the correlations for macro and micro-aggregates observed is in agreement with Tisdall and Oades (1982). These findings beg for more interest in understanding the effect of the endophytes in tall fescue and N management on soil structural stability.

## CONCLUSIONS

The direction and magnitude of the change in stable aggregates in a soil are influenced by the interaction of management, climatic and biological factors. Therefore, the effect of N management and forage species are also affected by the climatic and biological factors. The findings of this study indicate that the endophytes in the tall fescue did not affect macro-aggregates, but did affect micro-aggregates in the soil. The source of N and date of application affected both soil macro-aggregate and micro-aggregate stability. Early application of N as ammonium nitrate in September to stockpiled tall fescue in North Mississippi increased soil macro-aggregates, while urea increased soil micro-aggregates. Additional research across several locations and on different soil types are needed to determined the effect of management and climatic factors on aggregate stability.

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Table 1. Surface soil (0-15 cm) properties of Marietta loam at H.H. Leveck Animal Research Center, Mississippi State University.

| Property | Value |
|---|---|
| **Physical** | |
| Soil texture (%) | |
| Sand | 44 |
| Silt | 43 |
| Clay | 13 |
| **Chemical** | |
| pH | 6.2 |
| Ca (mg $kg^{-1}$) | 864 |
| Mg (mg $kg^{-1}$) | 37 |
| K (mg $kg^{-1}$) | 115 |
| P (mg $kg^{-1}$) | 62 |

Table 2. ANOVA result for water stable aggregates under six endophyte strains (ES) in tall fescue on a Marietta loam for 2 yrs. H.H. Leveck Animal Research Center, Mississippi State University.

| Source of variation | Water Stable Aggregate (%) | |
|---|---|---|
| | Macro-aggregates (>250 µm) | Micro-aggregates (125- 250µm) |
| | ----------------P value---------------- | |
| Block | | |
| ES | 0.1047 | 0.0134 |
| Date of application (D) | 0.9777 | 0.2956 |
| ES x D | 0.8587 | 0.7847 |
| Nitrogen Fertilizer (N)[1] | 0.3732 | 0.2809 |
| N x D | 0.0180 | 0.0001 |
| ES x N | 0.4032 | 0.6546 |
| ES x N x D | 0.6025 | 0.3553 |

[1]Two source of nitrogen, urea and ammonium nitrate and two application rates 56 and 112 kg N $ha^{-1}$, and control

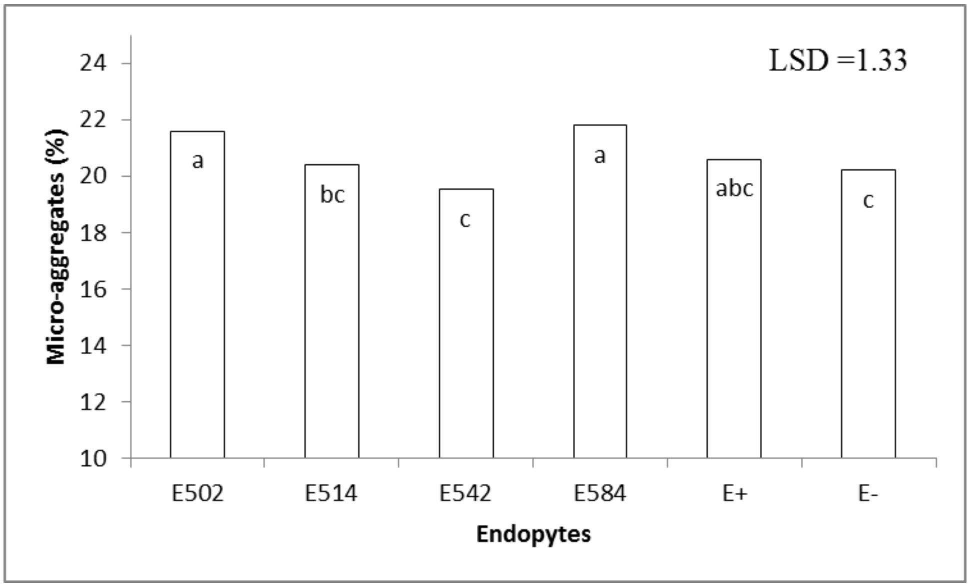


Fig. 1. Effect of tall fescue with endophytes [wild type (E+), novel (E502, E514, E542, and E584) and tall fescue without endophyte (E-)] under stockpiled condition on a Marietta loam at H.H. Leveck Animal Research Center, Mississippi State University. Values differed significantly when followed by different letters at $P < 0.05$.