Accomplishments (October 1, 2012 to September 30, 2013)
Reports from Arkansas and Kentucky only. No reports from UT, WI, NE OK, GA, ND.

Objective 1. Quantify N harvest efficiency of grassland agroecosystems by determining N inputs such as fertilizer, manure, atmospheric deposition and leguminous fixation and calculating N takeoff as food, fuel or fiber. No reports WI, OK, NE GA

Objective 2: Quantify the effect of dietary and animal factors on utilization and excretion of forage N by beef and dairy cattle. Investigators at AR evaluated the intake and digestibility of bermudagrass hay, dried distillers grains with varied degrees or caramelization.

Large round hay bales of predominantly bermudagrass with varied degrees of caramelization among and within bales were identified. Bales were labeled and core samples were taken from specific locations within the bales to validate visual appraisal of heat-damage with ADIN analyses. Hay from the bales was then separated into 3 levels of heat-damage (LOW, MED, HIGH) based on visual color. Fifteen non-pregnant, non-lactating Katahdin ewes (66.2 ± 5.17 kg initial BW) were stratified by weight within age and assigned randomly to 1 of the 3 treatments. Ewes were offered their respective chopped (2.5 cm) hays for ad libitum consumption through a 10-d adaptation period followed by 5-d of total fecal collection. On the final day of collection, blood samples were gathered via jugular venipuncture immediately prior to feeding and 4 and 8 h post-feeding and analyzed for concentrations of serum urea nitrogen (SUN). Dry matter intake, and digestibilities of DM, NDF, and ADF, as well as digestible DM and OM intake were greater ($P < 0.05$) from LOW and MED compared with those from HIGH. Organic matter digestibility and concentrations of SUN were greater ($P < 0.05$) from LOW compared with MED and from MED compared with HIGH ($P < 0.05$). Therefore, when provided as the only dietary choice, intake and digestibility of severely caramelized forage may be reduced compared with that of properly stored hay.

In addition, investigators at AR also investigated heat damaged dried distiller's grains (DDG) were also investigated as a dietary source.

Both conventional (CDDG) and a lower-fat DDG (LFDDG) were mixed 80:20 with water, placed in Al pans, covered with foil, and heated for either 3 or 5.5 h at 150C. Contents from the center of each pan were retained separately from the exterior 2 cm of each pan. The exterior 2 cm from the 5.5-h heating was charred excessively and retained to represent excessive heat damage (5.5CHR). Ruminally annulated beef cows (n=4; 533±14.0 kg BW) were offered bermudagrass hay with either no supplement or 0.45% of BW from either CDDG, LFDDG, or heated LFDDG (3 h at 150C) in a 4 × 4 Latin Square experiment. Nylon bags containing unheated CDDG and LFDDG, those heated for 3 or 5.5 h, and those from 5.5CHR were incubated in the rumen for multiple times up to 108 h. Dry matter remaining was fit to a non-linear model, resulting in estimations of immediately-soluble (A) and potentially degradable (B) fractions, along with disappearance rate (k_d). Cow diet did not affect ($P>0.29$) in situ parameters. Fraction B was greater ($P<0.05$), and k_d was less ($P<0.05$) from CDDG vs. LFDDG. Fraction B was greater ($P<0.05$) from 5.5CHR and DDG heated for 5.5 h, and k_d was slowest ($P<0.05$) from 5.5CHR followed by DDG heated for 5.5 h. Neither measure differed ($P>0.05$) between unheated DDG and DDG heated for 3 h. Linear relationships were strongest ($R^2>0.77$) between

acid-detergent insoluble N (ADIN, % of total N) and fraction A within DDG type. Linear and quadratic relationships between ADIN and other kinetic parameters were observed ($P<0.05$), but those relationships were lower ($R^2 \leq 0.41$) than for fraction A, and were even less ($R^2=0.06$ to 0.32) when analyzed across types of DDG. Therefore, moderate heat damage had little impact on ruminal DM kinetic measurements, and use of ADIN analyses to assess ruminal DM disappearance characteristics should be viewed with caution, even within DDG processing method.

AR also presented studies to determine if co-product feedstuffs could be used as the primary feed component to meet the energy demands for cows in late pregnancy.

Eighty-six crossbred cows (527 ± 0.8 kg BW) in late gestation were stratified by BW, BCS, and age and allocated randomly to either bermudagrass hay (8.8% CP, 72% NDF) offered ad libitum (BH; 3 reps.) or 6.4 kg of soybean hulls (SH; 3 reps) daily and allowed access to mixed-grass hay (3.0% CP, 82% NDF) for 1 h daily. All groups were held on 2-ha dormant bermudagrass pastures for 68 d. Changes in BW, BCS, and serum non-esterified fatty acids were minimal between treatments ($P \geq 0.31$). Birth weights tended ($P = 0.05$) to be greater from SH, but no difference was observed in dystocia scores ($P = 0.23$). In a companion study, 8 ruminally-fistulated cows (671 ± 32.0 kg BW) were stratified by BW and allocated randomly to 1 of 4 treatments in a 2-period study: limit-fed soybean hulls (LSH), limit-fed distillers' dried grains with solubles (LDG), a limit-fed mixture of soybean hulls and distillers' dried grains with solubles (MIX), or ad libitum mixed-grass hay (HAY; 10.6% CP, 71% NDF). Limit-fed diets were formulated to meet the ME requirements of an 11-mo post-partum mature beef cow. Diet amounts were increased over a 14-d period. Cows were then moved to indoor 3 × 4.3 m concrete pens fitted with rubber mats for a 14-d adaptation and 5 d of total fecal collection period. On the final day of collections, rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 hr post-feeding for measurement of rumen pH, VFA and NH_3-N concentrations. Dry matter disappearance was greatest for LSH and MIX, intermediate for LDG, and lowest for HAY ($P < 0.05$). A treatment × time interaction ($P < 0.05$) was observed for rumen pH, acetate, propionate, and butyrate concentrations, acetate: propionate ratio, and NH_3-N concentrations. Based on this information, it appears that co-product feedstuffs may be limit-fed to cows during late pregnancy without adverse effects on rumen fermentation, parturition or calf birth weights.

Objective 3: Evaluate fertilizer regimes, harvesting practices, and grazing management interactions with plant communities and soils emphasizing N capture and forage quality.

AR investigated the N turnover from three annual legumes followed by annual summer grasses and the performance of subterranean clover and white clover in bermudagrass-based grazing system.

Three annual legumes (crimson clover, arrowleaf clover, and hairy vetch) were no-till planted in a randomized complete block design. Seven treatments include 3 legume species and 4 nitrogen fertilizer treatments of teff which will be planted after the legumes have been terminated with an herbicide and incorporated into the soil via disking. The nitrogen treatments will include 0, 50, 100, and 150 kg ha^{-1} application rates. Teff will be harvested from these plots and those that were formerly covered with legumes. Regression will be used to correlate N-fertilization and

amounts of forage mass from teff which will allow the calculation of the potential N transfer from legumes to teff. Three subterranean clovers and two white clover varieties were no-till drilled into a bermudagrass pasture that was lightly disked or left untreated before planting. Crimson clover was used as control. A randomized complete block design with three replications was used for this experiment. Measurements will include but are not limited to seedling counts per unit area, forage mass in spring, and regrowth after grazing events during spring/summer.

Objective 4. Determine the fate of excess N in grassland agroecosystems by estimating key components of nutrient cycles including aqueous and gaseous N losses.

KY evaluated the effects of different tall fescue cultivars and endophyte presence and genotype on soil microbial processes and nutrient pools; and determined whether these effects were similar in both rhizosphere and bulk soils.

Tall fescue (*Lolium arundinaceum* (Schreb.)) is a cool-season perennial grass within which can live a fungal endophyte (*Neotyphodium coenophialum*) thought to provide enhanced edaphic and climactic stress tolerance to the host compared to non-infected neighbors. Our prior research demonstrated that a variety of root exudate compounds released from tall fescue were differentially affected by tall fescue cultivar, endophyte genotype and their interaction. Changes in root exudates could influence soil processes, including carbon and nitrogen pools. To test this, we collected rhizosphere and bulk soil samples from six year old field plots located in Lexington, KY planted with two different tall fescue cultivars (PDF and 97TF1), each containing four endophyte treatments [endophyte-free (E-) or infected with one of three strains of Neotyphodium (common toxic, novel AR542 and novel AR584)]. The influence of fescue cultivar, endophyte presence and strain, and soil sample location were assessed for soil organic carbon, soil total nitrogen, particulate and non-particulate organic matter (POM and n-POM, respectively) - C and – N, and dissolved organic carbon and nitrogen (DOC, DON) pools. Soil functional aspects were evaluated by measuring soil respiration using a unique oxygen sensitive microtiter plate method, as well as the activity of seven different soil enzymes related to C, N and P cycling. As expected, significant differences were found between rhizosphere and bulk soils in POM, n-POM, DOC, DON and enzyme activity; however, contrary to expectations, little discernible differences related to fescue cultivar or endophtye were found within either rhizosphere or bulk soils. These results suggest that the impact of endophyte presence and fescue cultivar on soil nutrient pools may not be detectable within a six year time period. It is also possible that the site-specific conditions, such as climate, hydrology, soil type, nutrient availability, or management, which reduced endophyte and cultivar effects on soil nutrient pools.

KY did another study to assess the controlling factors on aboveground biomass production and litter disappearance across a world-wide network of grassland sites.

Based on regional-scale studies, aboveground production and litter decomposition are thought to positively covary, because they are driven by shared biotic and climatic factors. Until now we have been unable to test whether production and decomposition are generally coupled across climatically dissimilar regions, because we lacked replicated data collected within a single vegetation type across multiple regions, obfuscating the drivers and generality of the association between production and decomposition. Furthermore, our understanding of the relationships

between production and decomposition rests heavily on separate meta-analyses of each response, because no studies have simultaneously measured production and the accumulation or decomposition of litter using consistent methods at globally relevant scales. Here, we use a multi-country grassland dataset collected using a standardized protocol to show that live plant biomass (an estimate of aboveground net primary production) and litter disappearance (represented by mass loss of aboveground litter) do not strongly covary. Live biomass and litter disappearance varied at different spatial scales. There was substantial variation in live biomass among continents, sites and plots whereas among continent differences accounted for most of the variation in litter disappearance rates. Although there were strong associations among aboveground biomass, litter disappearance and climatic factors in some regions (e.g. U.S. Great Plains), these relationships were inconsistent within and among the regions represented by this study. These results highlight the importance of replication among regions and continents when characterizing the correlations between ecosystem processes and interpreting their global-scale implications for carbon flux. We must exercise caution in parameterizing litter decomposition and aboveground production in future regional and global carbon models as their relationship is complex.

A third study by KY was to quantify the effects of fungal endophyte presence and genotype on pasture plant community composition, plant diversity, soil trace gas fluxes, and soil microenvironment and inorganic nitrogen concentrations.

Novel endophyte (*Neotyphodium coenophialum*) genotypes in symbiosis with tall fescue (*Lolium arundinaceum*) have been recently introduced to agricultural seed markets. These novel endophytes do not produce the full suite of toxins that the 'common toxic' form does, and therefore, may not have the same consequences on plant and soil processes. Here, we evaluated the effects of novel endophyte presence and genotype on ecosystem processes of tall fescue stands. We quantified the effects of the presence of the common toxic endophyte (CT), two novel endophyte genotypes (AR-542, AR-584), no endophyte (endophyte free, E-), and a mixture of all endophyte statuses (mix) within a single genotype of tall fescue (PDF) on various soil and plant parameters. Endophyte presence and genotype affected tall fescue cover and plant species richness: cover – CT, AR-542, AR -584, mix > E- and species richness – E-, mix > AR-542, AR -584 > CT. Most measured soil parameters had significant endophyte effects. For example, higher fluxes of soil CO2 and N2O were measured from stands of AR-542 than the other endophyte treatments. These results indicate that endophyte presence and genetic identity are important in understanding the ecosystem-scale effects of this agronomically important grass-fungal symbiosis.

These data illustrate that different fungal endophyte strains in symbiosis with tall fescue can have different effects on the surrounding plant community dynamics and belowground processes, including the flux of CO_2 and N_2O from the soil to the atmosphere. These effects should be considered in the environmental implications of producers adopting novel endophyte strains into their pasture systems.

What is the effect of native warm-season grass (NWSG) canopy development and characteristics on the establishment and production of forage legumes?

The establishment and persistence of legumes in grass mixtures is often constrained by their ability to exploit environmental niches within the stand. The leaf arrangement and canopy development varies greatly between native warm-season grasses (NWSG) species, and a closer examination of this variability in canopy architecture in relation to legume production may provide further insight into successfully maintaining these mixtures in sustainable forage-livestock systems. Five varieties of big bluestem [*Andropogon gerardii* Vitman] and six varieties of eastern gamagrass [*Tripsacum dactyloides* (L.) L.], indiangrass [*Sorghastrum nutans* (L.) Nash] and switchgrass [*Panicum virgatum* L.] were fertilized with 80 lbs N acre^{-1} or interseeded with either red clover [*Trifolium pratense* L.] or sericea lespedeza [*Lespedeza cuneata* (Dum. Cours.) G. Don]. Big bluestem, indiangrass, and switchgrass produced higher ($P < 0.05$) total forage yields than eastern gamagrass during the 2013 growing season. Incorporating legumes into NWSG stands did not increase ($P > 0.20$) forage yields relative to fertilized control. There was a significant ($P < 0.01$) interaction between NWSG species and the abundance of legume during the season. The percentage of red clover, as determined by a visual rating system, remained unchanged in the eastern gamagrass, whereas it declined in mixture with the other three grass species as the season progressed. The proportion of sericea lespedeza increased during later dates when interseeded into eastern gamagrass and switchgrass, but remained consistent throughout the season in big bluestem and indiangrass stands. The presence of sericea lespedeza was higher ($P < 0.01$) than red clover in the switchgrass stands at the end of the season, but was not different ($P > 0.18$) from the amount of red clover in big bluestem, eastern gamagrass, and indiangrass stands during the same period. Eastern gamagrass appeared to be compatible with either red clover or sericea lespedeza, and is most likely due to its lower ($P < 0.05$) leaf area index (LAI) and earlier harvest date which allowed more light to penetrate into the canopy. The more erect growth habit of sericea lespedeza allowed it to be more compatible with switchgrass than red clover. Although the percentage of sericea lespedeza was unchanged for big bluestem and indiangrass, the later harvest date allowed these species to maintain a maximized LAI longer into the season and may lead to poor persistence of both legume species. An additional year is needed to further evaluate the legume compatibility of these species. Genotypic effects for legume compatibility within a NWSG species were detected ($P < 0.08$) for switchgrass and indiangrass. However, these effects were primarily due to later maturing varieties allowing for higher initial legume percentages and no differences ($P > 0.30$) were detected in the varieties by the end of the season.

Impacts.

Carmelized hay is less desirable forage for Katahdin ewes, so protection of forages from heat denaturation is critical for better intake and digestibility.

Co-product feedstuffs, that is less expensive than regular feed and supplements can be substituted and limited-fed to cows during late pregnancy without adverse effects on rumen fermentation, parturition or calf birth weights.

Contrary to previous research, few differences were observed between tall fescue cultivars and endophyte statuses in this study. This research suggests that fescue-endophyte effects on belowground processes are complex, likely influenced by a variety of site-level characteristics, and are worthy of additional study.

Abiotic factors controlling grassland biomass production and litter decomposition were found to vary across regions of the globe, in contrast to the prevailing way that these two ecosystem parameters are handled in current day biogeochemical models. This result suggests that refinement of the current models might improve their predictive capability.

The selection of NWSG varieties had little effect on the persistence of legume species and legume compatibility was more determined based NWSG species and legume growth habit. Eastern gamagrass and switchgrass may provide producers opportunities with interseeding forage legumes into these stands.

Publications

Refereed Journal Articles/Book Chapters:

Caldwell, J. D. K. P. Coffey, J. A. Jennings, D. Philipp, A. N. Young, J. D. Tucker, D. S. Hubbell, III, T. Hess, M. L. Looper, C. P. West, M. C. Savin, M. P. Popp, D. L. Kreider, D. M. Hallford, and C. F. Rosenkrans, Jr.. 2013. Performance by spring and fall-calving cows grazing with full access, limited access, or no access to *Neotyphodium coenophialum*-infected fescue. J. Anim. Sci. 91:465-476.

Caldwell, J. D. D. Philipp, K. P. Coffey, L. A. Hardin, A. E. Bass, , A. N. Young, R. T. Rhein, and W. K. Coblentz. 2013. Intake and digestibility by sheep, *in-situ* disappearance iannulated cows, and chemical composition of crabgrass hayed at two moisture concentrations and treated with a non-viable *Lactobacillus*-lactic acid preservative. Anim. Feed Sci. Technol. http://dx.doi.org/10.1016/j.anifeedsci.2013.09.002.

Coblentz, W. K., K. P. Coffey, A. N. Young, and M. G. Bertram. 2013. Storage characteristics, nutritive value, energy content, and in vivo digestibility of moist large-rectangular bales of alfalfa-orchardgrass hay treated with a propionic-acid based preservative. J. Dairy Sci. 96:2521-2535.

Montgomery, T. G., K. P. Coffey, W. K. Coblentz, P. B. Francis, , W. A. Whitworth, and K. J. Bryant, 2013. Growth-performance of heifers grazing wheat and ryegrass sod-seeded into bermudagrass pastures following different tillage intensities on different seeding dates. Online. Forage and Grazinglands doi:1094/FG-2013-0226-01-RS.

Iqbal, J., J.A. Nelson, and R.L. McCulley. 2013. Fungal endophyte presence and genotype affect plant diversity and soil-to-atmosphere trace gas fluxes. Plant and Soil 364:15-27.

O'Halloran, L.R., E.T. Borer, E.W. Seabloom, A.S. MacDougall, E.E. Cleland, R.L. McCulley, S. Hobbie, W.S. Harpole, N.M. DeCrappeo, C. Chu, J.D. Bakker, K.F. Davies, G. Du, J. Firn, N. Hagenah, K.S. Hofmockel, J.M.H. Knops, W. Li, B.A. Melbourne, J.W. Morgan, J.L. Orrock, S.M. Prober, and C.J. Stevens. 2013. Regional contingencies in the relationship between aboveground biomass and litter in the world's grasslands. PLOS One 8(2):e54988.

Young, C.A., D. Hume, and R.L. McCulley. 2013. Fungal endophyte of tall fescue and perennial ryegrass: pasture friend or foe? Journal of Animal Science 91:2379-2394.

Published Abstracts:

Goff, B.M., L.C. Harris, G.O. Olson, and S.R. Smith. 2013. Effect of native warm-season grass canopy development and characteristics on the establishment and production of forage legumes. ASA-CSSA-SSSA Annual Meetings. Tampa, FL. Nov. 3-6.

Goff, B.M., C.E. Timberlake, E.K. Langlois, M.P. de Kanter, and L.C. Harris. 2013. Incorporating legumes into teff as an emergency source of forage. ASA-CSSA-SSSA Annual Meetings. Tampa, FL. Nov. 3-6.

Goff, B.M., G.E. Aiken, W.W. Witt, P.L. Burch, and E.S. Flynn. 2013. Steer and tall fescue pasture responses to grazing intensity and chemical seedhead suppression. In Proc. American Forage and Grassland Conference, Covington, KY. Jan. 6-8.

Guo, J., D.H. McNear, Jr., and R.L. McCulley. 2013. Alteration of carbon and nitrogen pools in bulk and rhizosphere soil in response to tall fescue cultivar and endophyte status. ASA-CSSA-SSSA Annual Meeting, Tampa, Florida, November 2013.