ABSTRACT

GLENNON, HEATHER. Effects of Adding Clovers to Tall Fescue Pastures on the Nitrogen Status of Animals, Forages and Soils. (Under the direction of Dr. Jean-Marie Luginbuhl).

Rotational grazing studies with Boer-cross and Kiko-cross meat goats (*Capra hircus*) were conducted during spring (March – May) 2012, 2013 and 2014 and fall (September – November) 2012. The objectives included evaluating animal performance, forage production, forage nutritive value, biological nitrogen fixation, and soil N status in tall fescue (*Lolium arundinaceum* Shreb.; TF) and TF pastures mixed with red clover (*Trifolium pratense* L.; RC) or white clover (*T. repens* L.; WC). The experiment was a split block design with 4 main nitrogen (N) treatments: TF mixed with red clover (TFRC), TF mixed with white clover (TFWC), TF fertilized with 112 kg N ha\(^{-1}\) annually (TFPOS) and TF with no additional N (TFNEG). Within each main treatment plot, there were grazed (G) and mowed (M) subplots. Nursing does and their twin kids grazed for 56 d each spring and were moved to new forage every 2 d. Eight month old wethers were grazed for 39 d only in fall 2012. \(^{15}\)N Natural Abundance technique was used to calculate %N derived from the atmosphere (%Ndfa) of clovers. Soil samples were taken before and after grazing/mowing and weekly thereafter and analyzed for nitrate-N (NO\(_3\)-N) and ammonium-N (NH\(_4\)-N). The proportion of forage dry matter (DM) as WC was similar in spring 2012 and 2013 but declined in 2014 (40.4, 43.2 and 18.7%, respectively). The proportion of forage DM as RC also declined as the trial progressed from 2012 to 2014 (30, 11.2 and 2.9%, respectively). There was less clover growth in the fall as compared to the spring \((P < 0.01)\). Mowed plots had a greater percentage of clover in 2012 and 2013 compared to grazed plots. Forage DM yield was affected by N treatment with TFNEG producing less \((P < 0.01)\) in spring and
TFWC producing less ($P < 0.01$) in fall than all other treatments. TFWC produced the greatest amount of forage N during spring ($P < 0.01$), but TFPOS produced the greatest amount of forage N during the fall ($P < 0.01$). The % Ndfa of TFWC declined from 82% to 42% from spring to fall and was correlated to an increase in soil NO$_3$-N levels ($P < 0.05$). TFWC had greater soil NH$_4$-N compared to the TF-only treatments in two of the three years and greater soil NO$_3$-N in each of the three years. Grazed treatments had greater inorganic soil N than mowed plots. Swards containing clover had lower C:N ratios which likely led to greater N mineralization and soil N levels even in the mowed treatments. TFWC fixed a total of 121 and 76 kg N ha$^{-1}$ in 2012 and 2013, respectively. TFRC fixed 62 kg N ha$^{-1}$ in 2012 but only 15 kg N ha$^{-1}$ in 2013. Average daily gain of the kids and wethers did not differ in spring (avg = 135.3 g/d; $P = 0.19$) or fall (avg = 71.2 g/d; $P = 0.20$). Animal output (kg gain ha$^{-1}$) was greater ($P < 0.01$) in the TFWC treatment compared to TFPOS and TFNEG during each of the three spring grazing seasons. Addition of clovers to the TF swards led to increased serum urea N and fecal N concentration of the does during the first 2 years. Inclusion of WC or RC in TF swards resulted in similar forage yields as in TFPOS and in greater animal output when the percentage of clover was $\geq 30\%$. Although careful grazing management of TF-clover stands can reduce or eliminate the need for N fertilization, reseeding clover every 2 to 3 years may be necessary to maintain optimal levels of clover DM in the stand.
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Effects of Adding Clovers to Tall Fescue Pastures on the Nitrogen Status of Animals, Forages and Soils

by
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DEDICATION

“To all the goats I’ve ever loved before”
BIOGRAPHY

Heather Mary Glennon, the daughter of Robert and Mary Lou Glennon was born on August 2, 1975, in College Township, Pennsylvania. She grew up on small farms in Pennsylvania, Maryland and Florida and received her first dairy goat at age seven. Heather loved goats so much that she kept adding as many animals to her herd as her parents allowed.

Heather graduated from Delaware Valley College in Doylestown, PA in May 1997 with a B.S. degree in Large Animal Science. She worked for Penn State Cooperative Extension for 4 years as a 4-H/Agriculture Agent in Bucks County. Heather had always dreamed of working with goats in a research setting, so she moved to North Carolina in 2001 to begin working on her Master’s Degree under Jean-Marie Luginbuhl. While working on her degree, Heather became the research technician for the North Carolina State University Meat Goat Program. She received her M.S. Degree in Animal Science in May 2004. Heather still owns a herd of meat goats and a flock of laying hens with her husband Jay, and they plan to continue raising grassfed livestock for many years.
ACKNOWLEDGMENTS

Many people have helped me get to this point in my academic career. First and foremost, I would like to thank my parents for pushing me to excel in school and for letting me raise as many animals as the barn could hold. Secondly, I would like to thank my supervisor and mentor, Dr. Jean-Marie Luginbuhl, for teaching me how to conduct great research and for being such a good-natured person. I was very fortunate to find the best graduate committee in the world, and they have given me a tremendous amount of positive support and critique. Of course I can’t forget my best friend April who has been at my side through every twist and turn of the past 14 years and even helped me sample goats when she was pregnant. Lastly, I would like to thank my husband Jay taking such good care of me and for making me laugh every day.
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CHAPTER 1

Effects of Legumes on Grazing Livestock, Forage Plants and Soil Nitrogen Within the Pasture Ecosystem: A Review

Abstract

Inclusion of legumes in grass pastures has the potential to benefit the livestock, plants and soil within these managed ecosystems. Grass-legume mixtures have been shown to increase livestock daily rate of gain through increased nutritive value of the forages and increased dry matter intake, while transfer of biologically fixed nitrogen (N) from legumes to companion grasses may also reduce the need for synthetic N fertilizers. As a multitude of ecosystem level benefits may exist for including legumes in pastures, quantifying the contribution of biological nitrogen fixation (BNF) remains a challenge. The excreta of ruminants grazing legumes will increase inorganic soil N, which then alters BNF and legume proportion in the sward. Unfortunately, this increase in soil N may also lead to an increase in environmental losses of nitrogen through volatilization and leaching. The lack of persistence and reliability of legume growth from season to season has been an additional challenge.

Many factors contribute to annual N fixation rates such as cyclical mineralization of soil N, weather and forage growth. While much research has been conducted studying grass-white clover (*Trifolium repens* L.) mixtures in Australia, New Zealand and Europe, less has been reported in the United States. This review describes the advantages and challenges of incorporating legumes into temperate pastures for US livestock producers. It also highlights areas of future research needed for more efficient N cycling within pasture ecosystems.
Introduction

Incorporating legumes into grass pasture systems as a low-input nitrogen (N) source is not a new concept, but is regaining popularity. Grass-legume pasture mixtures were popular in the US in the early 1950’s before the price of synthetic N fertilizer decreased, stimulating increased prevalence of fertilized grass monocultures (Nelson and Burns, 2006). The comeback of forage legumes is due in part to the recent trends towards low-cost intensively managed grazing systems, as well as ecological farming in the US. In the past two decades, grassland systems in Europe have undergone considerable changes due to societal and environmental pressures (Luscher et al., 2014). Changes in agricultural policy including a Nitrates Directive have led producers in the European Union (EU) to reevaluate the use of forage legumes in place of synthetic N fertilizer in an effort to protect water quality (Rochon et al., 2004). Most currently, the EU is supporting four initiatives (MultiSward, LegumeFutures, Animal-Change and LegumePlus) to help better understand the role legumes play in pasture ecosystems (Luscher et al., 2014). It has been a longstanding common practice in Australia and New Zealand to graze sheep (Ovis aries) and cattle (Bos taurus) on mixed grass-legume pastures (Peoples and Baldock, 2001; Ulyatt, 1971). The ley farming system in Australia has centered around rotating pasture legumes, mainly annual medics (Medicago spp.) and subterranean clover (Trifolium subterraneum), with grain crops for the past 40 to 50 years (Martin, 1996).

Legumes have the ability to convert atmospheric N$_2$ into plant available N due to their synergistic relationship with soil rhizobia bacteria via the process of Biological Nitrogen Fixation (BNF). Rhizobia bacteria (supplied either through seed inoculation or native soil
populations) colonize legume roots to form nodules where $N_2$ fixation occurs. The legume plant supplies carbohydrates to the bacteria, and the rhizobia in turn supply the legume with readily available $NH_3$. The amount of fixed $N$ that legumes contribute to a pasture ecosystem has been documented to range from 13 to 682 kg $N$ ha$^{-1}$ yr$^{-1}$ in mowed systems and 55 to 296 kg $N$ ha$^{-1}$ yr$^{-1}$ in grazed systems (Ledgard and Steel, 1992) possibly reducing or eliminating the need for synthetic $N$ in grass-legume mixed swards (Elgersma and Hassink, 1997). The $N$ fixed in roots and plant tissue (stolons) below the grazing height is rarely measured when calculating BNF, but has been shown to account for an additional 70% of fixed $N$ in white clover (Jorgensen and Ledgard, 1997).

There are several pathways that fixed $N$ can take to increase $N$ cycling in pastures. Ruminants excrete approximately 75 to 90% of their $N$ intake back onto pastures through urine and feces with urine accounting for 75% of the total $N$ excreted (Whitehead, 1995). Nitrogen can also be recycled back into a pasture ecosystem through the decomposition of the legume’s shoots, roots and nodules. The amount of $N$ transferred through root and nodule decomposition can range from 2 to 26% of BNF in mixed pastures (Ledgard and Steele, 1992).

Due to a higher tissue $N$ concentration in legumes compared to grasses, legumes have the potential to increase animal performance and sward quality when used as an alternative to $N$ fertilizer. Unfortunately, legume production and persistence in mixed swards have been reported as inconsistent and unreliable (Vipond et al., 1997). A tremendous amount of research has been conducted on white clover (Trifolium repens L.), arguably the most
important forage legume in temperate pastures, and its use in mixed swards partly due to its superior adaptation to grazing pressure.

The first objective of this paper is to review the impact of legumes, predominantly white clover, in mixed stands on animal performance, forage yield and quality, soil N and environmental N losses. The second objective is to review the factors affecting BNF in a pasture system and indicate potential areas of research which might increase the use of N fixing legumes in temperate pastures in the US.

**Effect of Legumes on Animal Performance**

Adding legumes to the grazing ruminant’s diet can improve live weight gain in sheep (Fraser et al., 2004; Marley et al., 2005), cattle (Beck et al., 2012; Yarrow and Penning, 2001) and goats (Turner et al., 2012). Lambs finished on monocultures of red clover (*Trifolium pratense* L.) or white clover had 45 to 65% greater daily gains than those grazing perennial ryegrass (*Lolium perenne* L.) and achieved optimal market weight in fewer days (Fraser et al., 2004, Marley et al., 2005). Other research has reported varied lamb performance on grass-legume mixtures. Nursing lambs on perennial ryegrass-white clover pastures had a greater rate of gain (38 to 49 g d⁻¹) than those on grass only with differences not carrying over from post-weaning to market weight (Vipond et al., 1993; 1997). Lambs grazing grass-red clover have been shown to have a 15 to 25% greater rate of gain than lambs grazing grass-white clover or grass pastures alone (Graves et al., 2012). Researchers in the UK comparing perennial ryegrass-white clover pastures to ryegrass swards fertilized with 400 kg N ha⁻¹ reported no difference in daily gains of sheep (Orr et al., 1990).
Cattle can also benefit from the addition of legumes into pastures. Steers grazing perennial ryegrass-white clover gained 0.13 to 0.18 kg d\(^{-1}\) more than those grazing ryegrass alone (Yarrow and Penning, 2001). Steers grazing tall fescue (*Lolium arundinaceum* Shreb.)-white clover have been shown to have a greater live weight gain (+ 0.2 kg d\(^{-1}\)) during the spring grazing season, although no difference was seen in total gain/ha (Beck et al., 2012). Similarly, stocker cattle grazing the annual legume mixture (arrowleaf clover [*Trifolium vesiculosum*], field pea [*Pisum sativum* L.] and hairy vetch [*Vicia villosa* Roth.]) sown into ryegrass or tall fescue showed similar animal gains and total gain ha\(^{-1}\) as those grazing grass that had been fertilized with 58 to 74 and 103 kg N ha\(^{-1}\), respectively (Butler et al., 2012; Interrante et al., 2012).

Interseeding white clover into fungal-infected KY 31 tall fescue to dilute some of the negative effects of fescue toxicosis has been a common recommendation to US livestock producers. Research has shown increased weight gain for cattle grazing infected fescue-clover mixes versus monocultures (Chestnut et al., 1991; McMurphy et al., 1990). Because tall fescue is predominant in central and eastern United States, more grazing research on fescue-legume stands utilizing different livestock and legume species would be beneficial.

Very little research has been published concerning goats (*Capra hircus*) grazing grass-legumes mixtures, with only one published account of legume effects on meat goats. Turner et al. (2012) documented an increase in daily gain (+ 34g d\(^{-1}\)) of goats grazing grass-red clover over those grazing orchardgrass (*Dactylis glomerata* L.) monocultures two out of three years.
Increased animal performance on grass-clover swards is likely linked to greater voluntary dry matter (DM) intake and forage nutritive value due to the legumes’ higher crude protein (CP) and lower fiber concentrations as compared to grasses (Rochon et al., 2004; Ulyatt, 1971). White clover has lower neutral detergent fiber (NDF) values, faster particle breakdown and a higher rate of passage through the rumen than grasses (Jamot and Grenet, 1991). Several grazing trials have reported increased DM intake of ruminants grazing a mixed sward that contained clover over those grazing grass alone (Fraser et al., 2004; Orr et al., 1990). Sheep grazing ryegrass-white clover selected a diet higher in clover content, 40% higher in N concentration and 10% higher in-vitro organic matter digestibility than the herbage on offer in the pasture (Curll et al., 1985). Likewise, Ulyatt (1971) reported sheep grazing white clover had greater intake of N and readily fermentable carbohydrate than those grazing perennial ryegrass.

**Effect of Legumes on Pasture Swards**

**Sward Yield**

The addition of white clover to grass stands has been reported to reduce annual forage production and consequently animal stocking rates when compared to fertilized grass monocultures (Beck et al., 2012; Turner et al., 2012; Yarrow and Penning, 2001). Dry matter yields of ryegrass-white clover swards have been measured at 80, 83 and 77% of the yields of ryegrass fertilized with 160, 210 and 420 kg N ha⁻¹, respectively (Curll et al., 1985; Orr et al., 1990; Vipond et al., 1997). The stocking rate of ewes and twin lambs grazing perennial ryegrass-white clover has been calculated at 63%, 77% and 82% of ryegrass swards fertilized...
with 160, 420 and 190 kg N ha\(^{-1}\), respectively (Orr et al., 1990; Vipond et al., 1993; 1997). Grass-red clover pastures have been found to only maintain 60% of the stocking rate of orchardgrass pastures fertilized with 56 kg N ha\(^{-1}\) when growing goats grazed during the summer (Turner et al., 2012). This is in contrast to the work done by Vines et al. (2006) that found tall fescue over-seeded with red clover produced a similar annual yield to tall fescue fertilized with 180 kg N ha\(^{-1}\). Comparing legume species under grazing conditions, ryegrass-white clover mixtures had greater DM yields than red clover, alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.)-ryegrass mixtures (Kleen et al., 2011).

Even at a reduced stocking rate, similar animal output (kg liveweight ha\(^{-1}\)) can be achieved by animals grazing grass-clover mixtures due in part to increased daily gain (Vipond et al., 1993). When the white clover proportion of a sward was 15 to 20%, Vipond et al. (1997) reported animal output to be similar to animals grazing N fertilized grass pastures. Rochon et al., (2004) recommend white clover should comprise 30% of the DM in a grass pasture in order to maximize pasture yield and animal performance, whereas Thomas (1992) reported pastures with high utilization (50-70%) need 35 to 45% legume DM to balance N input requirements.

*Sward Quality*

Swards containing legumes generally have higher nutritive values than grass monocultures. Yarrow and Penning (2001) found that as the proportion of white clover in perennial ryegrass pastures increased, the N concentration of the sward also increased with an observed decrease in water soluble carbohydrate (WSC). Sugar content and CP of forages have an inverse relationship, possibly because WSC are used by legumes for protein.
synthesis (Almodares et al., 2009; Keim and Anrique, 2011). Butler et al. (2012) found a mixture of annual legumes (arrowleaf clover, field pea and hairy vetch) were higher in CP and lower in NDF than their rye-annual ryegrass counterparts. The CP and \textit{in-vitro} dry matter digestibility (IVDMD) concentrations of kura clover (\textit{Trifolium ambiguum}), alfalfa and birdsfoot trefoil monocultures were higher than orchardgrass, smooth brome grass (\textit{Bromus inermis} Leyss.) and intermediate wheatgrass (\textit{Thinopyrum intermedium}) (Sleugh et al., 2000). Comparing legume species under grazing conditions, ryegrass-white clover mixtures had similar N yields as ryegrass-red clover and ryegrass-alfalfa (Kleen et al., 2011).

Sowing legumes into grass pastures can also influence the nutritive value of the grass. Researchers estimated apparent N transfer from clover to grass ranging from 29 to 113 kg N ha\(^{-1}\) in mowing trials when no additional N fertilizer was added and 96 to 132 kg N ha\(^{-1}\) when mixtures were fertilized with 150 to 180 kg N ha\(^{-1}\) (Elgersma et al., 2000; Elgersma and Hassink, 1997). White clover in mixed swards increased the N concentration of the companion grass compared to unfertilized grass monocultures and increased the total N yield of the sward when compared to mixtures fertilized at 150 to 180 kg N ha\(^{-1}\). In contrast, both orchardgrass and ryegrass fertilized at 224 kg N ha\(^{-1}\) had greater herbage N accumulation than those grasses mixed with either alfalfa or white clover (Stout et al., 2000). Nitrogen inputs such as animal excreta or fertilizer to grass-legumes mixtures will increase the N concentration of ryegrass in mixtures, but generally does not impact the N concentration of the clover (Vinthor 1998; Eriksen and Hugh-Jensen, 1998; Ledgard et al., 2001).
Effect of Legumes on Soil Nitrogen Cycling

Pasture soil N concentrations are very cyclic throughout the year. Mineral soil N is lower during the spring season due to the high demand by rapidly growing forages for N and greater in the fall and early winter as forage growth declines (Peoples and Baldock, 2001). Initial increases in soil N result from grazing ruminants recycling plant material via excreta. Two studies demonstrated this rapid N flux following a single application of urine at 441 or 746 kg N ha\(^{-1}\) with maximum soil ammonium (NH\(_4\)) and nitrate (NO\(_3\)) levels measured within 3 days and 10 to 20 days, respectively (Vinther, 1998; Menneer et al., 2003). Inorganic soil N values returned to background levels within 4 to 23 weeks (Vinther, 1998; Menneer et al., 2003). There is limited research reporting the direct effect of animals grazing legumes on inorganic soil N values. Vines et al. (2006) found soil NH\(_4\)-N levels were higher, but soil NO\(_3\)-N levels were lower in fescue-red clover pastures compared to tall fescue pastures fertilized with 180 kg N ha\(^{-1}\).

Decomposition of legume plant tissues can also increase soil N values. Soil N mineralization rates of mixed grass-legume pasture residues are greater than rates of grass monocultures. Mineralization is the process whereby soil microorganisms convert organic N contained within soil organic matter into inorganic forms the plant can use (NH\(_4\) and NO\(_3\)). Because legumes have an elevated N concentration, soil organic matter C:N ratios are lower, which can increase N mineralization (Elgersma and Hassink, 1997; Loiseau et al., 2001). Ranells and Wagger (1996) reported greater N concentrations and lower C:N ratios of rye – hairy vetch (Secale cereal - Vicia villosa) cover crop mixtures compared to rye monocultures (C:N 14-21:1; 40:1, respectively) resulting in a greater amount of N released during 8 wk of
decomposition (108 vs 24 kg N ha\(^{-1}\), respectively). Nitrogen mineralization following an annual legume-based pasture can range from 60 to 100 kg N ha\(^{-1}\) depending on soil fertility (Peoples and Baldock, 2001). The sloughed-off root cells of pasture legumes can also be an important soil N source. In a mowing study, rhizodeposition of N compounds from red clover and white clover accounted for up to 80% of plant derived N in the soil (Hogh-Jensen and Schjoerring, 2001).

Effect of Legumes and Grazing Animals on N Loss to the Environment

There are several ways N can be lost from a pasture ecosystem to the environment. Ammonia (NH\(_3\)) and nitrous oxide (N\(_2\)O) are lost as gasses into the atmosphere, whereas nitrate is lost through leaching in the soil below the root zone and to ground water. Losses will vary based on animal excretal N, precipitation, temperature, soil type and inorganic soil N levels.

Ruminants grazing forages high in N, especially pastures containing white clover, are known to have low nitrogen utilization efficiency (NUE) due to rapid plant protein degradation in excess of rumen microbes’ ability to assimilate it resulting in a large proportion of N-intake being excreted in urine and feces (Kleen et al., 2011). When not captured by the microbes, excess ruminal NH\(_3\) is absorbed across the ruminal wall and converted to urea in the liver. Urea is then recycled back to the rumen, used by the mammary gland or excreted in urine. Marini and Van Amburgh (2003) reported linear increases in urinary urea of dairy cattle as dietary intake N increased from 1.45% to 3.4%, while fecal N was not affected. Turner et al. (2012) reported that the total digestible nutrients
(TDN)/CP ratios of grass and grass-legumes pastures managed for high nutritive value were less than the recommended 5.8 to 6.1 for growing meat goat kids. The ratios correlated with high blood urea nitrogen (BUN) levels suggesting animals were not utilizing forage protein efficiently.

Once excreted, urine urea is hydrolyzed into ammonia and can be lost from the system by volatilization during or immediately after deposition (Fillery, 2001). Urine urea can account for 57 to 77% of cattle urine N (Huntington and Archibeque, 1999) or 80 to 90% of sheep urine N (Sherlock and Goh, 1984). Whitehead (1995) reported that within 2 to 3 days after grazing, 4 to 38% of urinary N can be lost to volatilization. Other researchers have documented volatilization losses of 20 to 27% after initial urine application, with an increase up to 30 and 38% after a second and third urine application, respectively (Vinther 1998; Sherlock and Goh, 1984). Volatilization losses of 15 to 17 kg N ha\(^{-1}\) were documented on dairy farms in New Zealand in grass-clover pastures (Ledgard et al., 1999). Dry, hot conditions favor increased volatilization (Sherlock and Goh, 1984; Eckard et al., 2003) with urine losing 30 to 50% N through volatilization during dry, warm months when forage growth is low (Fillery, 2001).

Agricultural production has been linked to increased nitrous oxide (N\(_2\)O) emission, a major greenhouse gas due to its long residency time of approximately 100 years in the atmosphere and global warming potential of 296 times greater than carbon dioxide (NRC, 2003; IPCC, 2001). Nitrous oxide is lost during the denitrification process which relies on high levels of soil NO\(_3\)-N and anaerobic conditions. Whereas these losses are generally low within pasture ecosystems, the potential for N\(_2\)O loss is increased with forages that have low
C:N and lignin:N ratios such as legumes (Gomes et al., 2009). Field studies in New Zealand reported losses of 3 to 7 kg N ha\(^{-1}\) from grass-clover swards (Ledgard et al., 1999). Soil N\(_2\)O emissions from legume cover crops were reported to be greater than from grass cover crops, although losses only represented 0.39 to 0.75% of the total legume N (Gomes et al., 2009). In that study, maximum N\(_2\)O emission was only 1.3 kg N ha\(^{-1}\) yr\(^{-1}\) and directly related to N inputs by legume biomass. Laboratory studies have reported higher losses of up to 55 kg N ha\(^{-1}\) when environmental conditions were maintained at optimal levels (deKlein and Logstestijn, 1994).

Nitrate leaching is another major pathway for N to be lost to the environment in a pasture ecosystem with mineralization of animal excreta being the primary source (Cuttle et al., 1998; Eriksen et al., 2004). A single urination on average can deliver 200 to 800 kg N ha\(^{-1}\) to patches in the pasture (Snow et al., 2013), which far exceeds the plant’s nutritional requirement and can result in significant leaching (Ledgard and Saunders, 1982). Eriksen et al. (2004) reported greater nitrate leaching on grazed ryegrass-clover swards than mowed ryegrass-clover swards due to greater localized urine and feces deposition in grazed swards. The authors suggested a combination of grazing and mowing to reduce the amount of excess N subject to leaching.

In grass-legume pasture systems, the uptake of soil nitrate by companion grasses can serve to reduce inorganic soil N levels and thereby decrease the amount of nitrate lost from the system via leaching. The work of Loiseau et al. (2001) reported leaching under grass-clover swards was less than under pure white clover stands (1 to 19 kg N ha\(^{-1}\) vs 28 to 140 kg N ha\(^{-1}\), respectively). Others have reported nitrate leaching losses ranging from 5 to 34 kg N...
ha\(^{-1}\) with sheep grazing grass-clover pastures (Cuttle et al., 1998; Ruz-Jerez, 1995) to 20 to 74 kg N ha\(^{-1}\) with dairy cattle grazing grass-clover pastures in New Zealand (Ledgard et al., 1999).

Leaching is accelerated when soil NO\(_3\)-N levels accumulate before periods of high rainfall and/or when the soil texture is sandy (Cuttle et al., 1998; Fillery, 2001). Nitrate leaching has been shown to account for 8 to 32\% of the total N fixed by legumes, with the highest values occurring during wet periods (Ledgard et al., 1999; Ridley et al., 2001). Stout et al., (2000) found that leachate NO\(_3\)-N concentration from grass-legume pastures in the Northeast US ranged from 9.2 to 15.8 mg l\(^{-1}\) during a drought year exceeding the United States Environmental Protection Agency’s limit of 10 mg l\(^{-1}\) nitrate in drinking water. The death of legume nodules during the hot, dry summer months can possibly cause the increase in leachate nitrate concentration during the rainy season.

Caution should be used, however, when analyzing results of nitrate leaching studies. Increased N input whether through fertilization or biological fixation by legumes may increase DM production resulting in increased animal stocking rates and excretal N inputs which can lead to greater nitrate leaching (Cuttle et al., 1998). That study found a positive correlation of the amount of leached nitrate to the proportion of clover in the stand possibly due to an increase in stocking rate and increase in grazing days. Alternatively, as stands age, the proportion of clover will decline leading to decreased DM production, N fixation and nitrate leaching (Eriksen et al., 2004).

It is well understood that nitrogen fertilizer applications also contribute to N losses. The replacement of fertilizer by legumes as the N input in a pasture ecosystem may decrease
N losses, as legumes will limit the amount of fixed N as soil inorganic N levels rise (Ledgard et al., 2001). Gaseous N losses under fertilization (200 and 400 kg N ha\(^{-1}\)) have been shown to be higher than in grazed systems, ranging from 2 to 10 times greater when compared to grass-legume swards with no synthetic N (Ledgard et al., 1999). Urea fertilizer led to more volatilization losses especially during the summer, whereas ammonium nitrate fertilizer led to greater denitrification losses during the wet Australian winter compared to unfertilized grass-clover mixtures (Eckard et al., 2003). Leaching losses under grazed, N-fertilized ryegrass have been reported to be 7 to 10 times greater than under grass-legume systems (Ledgard et al., 1999; Eriksen et al., 2004; Ruz-Jerez 1995). Conversely, Stout et al. (2000) found leachate NO\(_3\)-N concentration was highest in grass-alfalfa swards, intermediate in grass-white clover swards, and lowest in grass monocultures fertilized with split N applications of 196 and 252 kg N ha\(^{-1}\) during a 2 year cutting trial. During a drought year when N will not be efficiently utilized by grasses, N fertilizer can be withheld resulting in decreased leaching, while nitrates from legumes would be subject to leaching regardless.

There are a several management practices that have been suggested to possibly help mitigate these N losses from pastures. They include utilizing forages with increased rumen undegradable protein (RUP) and WSC concentrations as well as increasing fermentable carbohydrates in the diet (Keim and Anrique, 2011). Condensed tannins in birdsfoot trefoil and sericea lespedeza (Lespedeza cuneata G. Don) and the enzyme, polyphenol oxidase, in red clover reduce protein degradation rates in the rumen leading to increased ruminal bypass protein and NUE of ruminants (Kleen et al., 2011; Luscher et al., 2014). A 42% decrease in urine urea and a 39% decrease in NH\(_3\) emissions were observed in dairy cattle fed a diet
lower in rumen degradable protein (Smits et al., 1995). Broderick (1995) recommended diets should include 35% RDP to increase protein efficiency. When dairy cattle grazed perennial ryegrass cultivars of differing WSC concentrations, an increase in milk N yield and decrease in milk urea N concentrations were reported in animals grazing forages with a greater WSC concentration, but NUE was not affected (Tas et al., 2006). Supplementing a high protein forage diet with fermentable carbohydrates has been reported to decrease ruminal ammonia, increase milk yield and decrease urine urea, because energy from the carbohydrates allow the microbes to capture more rumen NH₃ which leads to a greater NUE (Fillery, 2001; Keim and Anrique 2011). Additional research is needed to capture more of the N cycling that takes place in legume-based pastures.

Factors Affecting Biological Nitrogen Fixation

Efficiency of BNF levels is dynamic throughout the year and can vary based on soil N status, weather and forage growth. During dry, hot periods, inorganic soil N has been shown to accumulate due to reduced forage growth resulting in a decrease in percentage of % N derived from the atmosphere (Ndfa) to levels below 50% (Hoglund and Brock, 1978; Ledgard et al., 1996). Vinther (1998) calculated half of the N was fixed within the first 2 to 3 months of the growing season. Ledgard et al. (2001) reported that white clover fixed 61 to 72% of its N during the spring and summer months. The amount of N fixed by a legume is largely dependent upon DM production and % N derived from the atmosphere (%Ndfa). Defoliation, excreta of grazing animals and N fertilization can impact BNF in the short and long-term (Harris and Clark, 1996, Ledgard, 2001; Meener et al., 2003).
Management Effects on Nodules.

Harvesting legume shoot material has been shown to induce a short term reduction in the BNF activity of nodules. Notably, a decrease in acetylene reduction was detected in white clover and alfalfa nodules within 24 hours after defoliation (Moustafa et al., 1969). Rates stayed low for 5 to 13 days, but then returned to control levels by day 18 to 21 (Moustafa et al., 1969; Vance et al., 1979). Following harvest, alfalfa nodules were also reported to senesce and lose starch and soluble protein concentration within 10 days (Vance et al., 1979). Current theories to explain this decrease in nodule function center around carbon and oxygen supply regulation and N feedback regulation (Schulze, 2004). A decrease in nodule mass has been measured in white clover plants following defoliation as well as N fertilization at 200 or 400 kg N ha\(^{-1}\) (Harris and Clark, 1996).

Grazing/Mowing Effects on Clover Proportion

The grazing behavior of sheep, cattle and goats heavily influences the clover content and persistence in a sward as well as subsequent animal performance. Sheep have a greater selectivity for clover than goats resulting in lower clover mass in the sward over time (Penning et al., 1996; del Pozo et al., 1996). Grazing goats ahead of lambs as well as co-grazing does with ewes resulted in twice as much white clover in the sward and increased lamb daily gain and gain ha\(^{-1}\) (del Pozo et al., 1996; Townsend and Radcliffe, 1990). Goats’ preference for woody browse species, weeds and grass lead to greater clover content in mixed swards (Clark et al., 1982). It is also possible that the clover type influenced selectivity as goats do not like to graze as close to the ground as other livestock species. Rotationally grazing beef cattle on grass-white clover also resulted in significantly more
clover content than with grazing sheep (13.5% vs 4.8%, respectively) over a 7 year study (Nolan et al., 2001). Reduced sward heights caused by a high stocking rate increased clover production by 30%, due to decreased competition between grasses and clover (Ledgard et al., 2001). Mowing ryegrass-clover swards at 4 cm vs 8 cm was shown to increase total DM by 16% and clover proportion by 31% (Frame and Boyd, 1987) possibly due to the increased light saturation boosting the number of clover growing points and photosynthetic activity. Rotational grazing as opposed to continuous grazing, especially with sheep, resulted in better clover persistence in the sward (Cuttle et al., 1998). Additional research on mixed species grazing and management practices can improve legume persistence in US pastures.

**Excreta Effects on Clover Proportion**

Animal excreta, particularly urine, have been reported to decrease the proportion of legumes present in mixed swards (Menneer, et al., 2003; Vinther 1998). The transfer of fixed N from legumes to grasses has been shown to lead to increased grass growth especially in the spring (Ledgard and Steele, 1992). Reported increases in annual grass production due to urine application ranged from 56 to 85% (Menneer, et al., 2003; Vinther 1998). Because, clovers do not compete well with the rapidly growing, taller grasses for light or inorganic soil N, legume content and/or production decreases (Ledgard and Saunders, 1982; Menneer et al., 2003). The proportion of clover in urine-treated ryegrass-white clover plots decreased to approximately 50% of control plots within 80 days after urine application (Vinther, 1998). Likewise, after application of cattle feces, the proportion of clover was reduced from 50% to 30% within 20 cm from the edge of the dung pat (Vinther, 1998). As grass growth slowed later in the season and soil N values declined, legume production and BNF increased.
(Ledgard and Steele, 1992; Menneer et al., 2003). Lack of excreta and decreased N input may explain why white clover proportion is greater under mowing vs grazing conditions (Kleen et al., 2011). The proportion of clover in mowed swards has been reported as double (40% vs 20%, respectively) the proportion in grazed treatments (Eriksen and Hogh-Jensen, 1998).

**Excreta Effects on percent Ndfa**

It is challenging to determine the effect of excreta on BNF because of its uneven distribution on pastures. As previously mentioned, ruminants recycle via excreta approximately 75 to 90% of their N intake back onto pastures with urine accounting for 75% of the total N (Whitehead, 1995). At a stocking density of 540 animal days ha\(^{-1}\) year\(^{-1}\), it has been calculated that grazing cattle distributed urine over 22% of a pasture area and feces over 5% of the area (Eriksen and Hogh-Jensen, 1998). Urine urea can rapidly hydrolyze into ammonium nitrogen, undergo nitrification to soil nitrate nitrogen and subsequently increase inorganic soil N values if not utilized. Excreta can lead to elevated inorganic soil N values which in turn decrease %Ndfa, because the clover preferentially utilizes soil N over fixed N, which requires more energy. Urine has been reported to decrease the % Ndfa of clover by 37 to 54% over a one month period (Eriksen and Hogh-Jensen, 1998; Vinther 1998). As low as a single application of urine has been shown to induce a decline in %Ndfa from 84% to 25% within 43 days with recovery of BNF to original levels taking almost a year (Menneer et al., 2003). The effect of feces on BNF is less than urine and has been reported at 20% reduction within 10 cm of the dung pat (Vinther, 1998). Fecal N must first be mineralized by soil microorganisms into
plant available N, whereas urine urea is rapidly transformed into ammonia for use in plants. The % Ndfa of mixed grazed plots was lower than mowed plots by 15 percentage points (Eriksen and Hogh-Jensen, 1998).

Depending on grazing method (rotational vs continuous) and stocking rate, 25 to 66% of a pasture can be affected by excreta every year resulting in a 10 to 38% decrease in total fixed N annually (Menneer et al., 2003; Vinther 1998; Ledgard and Steele 1992; Ledgard et al., 1996).

Fertilizer Effects on Clover Proportion and %Ndfa

The addition of N fertilizer to a grass-legume pasture will also increase inorganic soil N status and decrease BNF (Ledgard et al., 1996). Annual rates of fixed N decreased 36% and 68 to 75% after ryegrass-clover pastures were fertilized with 200 and 400 kg N ha⁻¹, respectively (Harris and Clark, 1996; Ledgard et al., 2001). When soil inorganic N exceeded 40 mg kg⁻¹, the % Ndfa dropped below 50% (Ledgard et al., 2001). Applying N fertilizer to grass-clover mixtures also affects the yield and the proportion of grass and clover as summarized in Table 1. Consequently, the total amount of fixed N will decrease due to this reduced clover yield (Ledgard and Saunders, 1982).

Effect of Cultivar on BNF

When conditions are cool and moisture is not limiting, legume DM production drives the total amount of fixed N (Ledgard, et al., 1996). There are mixed results regarding the best white clover variety for N fixation in pastures. Larger leaf, erect growing, more productive white clover cultivars such as ‘Kopu’ and ‘Alice’ have been shown to have a greater impact on annual fixed N amounts than those with smaller leaves and prostrate
growth, because their growth and persistence can withstand repeated external N inputs (Frame and Boyd 1987; Ledgard et al., 1996; Elgersma et al., 2000). Intermediate leaf type white clovers such as ‘Durana’ and ‘Patriot’ reportedly have better persistence under high grazing pressure in grass mixtures than large, erect ladino types due to their aggressive stolons (Bouton et al., 2005).

Summary

With the increasing emphasis on improving the sustainability of agricultural systems and interest in grass-fed livestock products, the time is right to reexamine the biology of grass-legume pasture mixtures to improve efficiency of resource use to assist producers in making sound management decisions. When managed correctly, legumes have the ability to replace some or all of the synthetic N inputs without a concomitant decrease in animal output. White clover’s superior nutritive value and ability to withstand grazing pressure have made it the focus of much research. Nevertheless, there are numerous other annual and perennial legumes yet to be examined in combination with the many species of grasses currently grown in temperate US pastures. It has been well established that incorporating white clover into tall fescue pastures can dilute some of the effects of fescue toxicosis. Unfortunately, stands grazed by cattle often lose much of the clover within a few years. Additional grazing management research utilizing different legume species, re-sowing strategies, co-species grazing, grazing heights and rest periods can help preserve the mixed stands. Research that focuses on improving ruminant NUE through dietary manipulation and
the use of forages with greater RUP looks promising to help decrease excretal N subject to environmental losses.

There is also a need for interdisciplinary research to better understand the cyclical/seasonal nature of soil N status and its effect on BNF. During periods of high forage growth, soil N is low while BNF remains high resulting in the majority of N being fixed during the spring and summer months. The increase in the rate and amount of forage N cycling in a pasture ecosystem primarily via animal urine and feces can lead to increases in N losses to the environment. More research is needed so producers can capture more of this excess soil N going into the wet winter months when forage growth is slow and thus improve N-use efficiency.
REFERENCES


Table 1.1 Effects of fertilizing grass-clover pastures on forage yield and proportion of clover in swards

<table>
<thead>
<tr>
<th>Forage Mixture</th>
<th>Fertilizer kg N/ha</th>
<th>Total DM Yield</th>
<th>Grass DM Yield</th>
<th>Clover DM yield</th>
<th>% Clover</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryegrass-white clover</td>
<td>150; 180</td>
<td>NR</td>
<td>+42%</td>
<td>-48%</td>
<td>-58%</td>
<td>Elgersma et al., 2000</td>
</tr>
<tr>
<td>Ryegrass-white clover</td>
<td>200; 400</td>
<td>+12%; +25%</td>
<td>NR</td>
<td>-21%; -60%</td>
<td>NR</td>
<td>Ledgard et al., 2001</td>
</tr>
<tr>
<td>Ryegrass-white clover</td>
<td>200; 400</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>-37%; -87%</td>
<td>Harris and Clark, 1996</td>
</tr>
<tr>
<td>Ryegrass-white clover</td>
<td>360</td>
<td>+33%</td>
<td>NR</td>
<td>-88%</td>
<td>-92%</td>
<td>Frame and Boyd, 1987</td>
</tr>
<tr>
<td>Ryegrass-white clover</td>
<td>390</td>
<td>+25%</td>
<td>+41%</td>
<td>-17%</td>
<td>NR</td>
<td>Ledgard et al., 1996</td>
</tr>
</tbody>
</table>

NR – results not reported
CHAPTER 2

Addition of clover to tall fescue pastures positively impacts forage characteristics and meat goat performance


Abstract

Rotational grazing studies with Boer-cross and Kiko-cross meat goats (*Capra hircus*) were conducted during spring (March – May) 2012, 2013 and 2014 and fall (September – November) 2012 to evaluate forage production and nutritive value as well as animal performance in tall fescue (*Lolium arundinaceum* Shreb.; TF) and TF pastures mixed with red clover (*Trifolium pratense* L.; RC) or white clover (*Trifolium repens* L.; WC). The experiment was a split block design with 4 main nitrogen (N) treatments: TF mixed with red clover (TFRC), TF mixed with white clover (TFWC), TF fertilized with 112 kg N ha⁻¹ annually (TFPOS) TF with no additional N (TFNEG). Within each main treatment plot, there were grazed and mowed subplots. Nursing does and their twin kids grazed for 56 d each spring and were moved to new forage every 2 days. Eight-month old wethers were grazed for 39 d in fall 2012 only. Additional put and take animals were used to equalize stocking density per available forage mass. The proportion of forage dry matter (DM) as WC was similar in spring 2012 and 2013 but declined in 2014 (40.4, 43.2 and 18.7%, respectively). The proportion of forage DM as RC also declined as the trial progressed (30,
There was much less clover growth in the fall as compared to the spring \((P < 0.01)\). Mowed plots had a greater percentage of clover in 2012 and 2013 than grazed plots. Forage DM yield was affected by N treatment with TFNEG producing less \((P < 0.01)\) in spring and TFWC producing less \((P < 0.01)\) in fall than all other treatments. Clover plants had greater N concentrations than fescue plants resulting in TFWC swards having the greatest crude protein (CP) concentrations during spring \((P < 0.001)\). Average daily gain of the kids and wethers did not differ in spring (mean = 135.3 g/d; \(P = 0.19\)) or fall (mean 71.2 g/d; \(P = 0.20\)). Animal output (kg gain ha\(^{-1}\)) was greater \((P < 0.01)\) in the TFWC treatment compared to both TFPOS and TFNEG during each of the three spring grazing seasons. Addition of clovers to the TF swards led to increased serum urea N and fecal N concentrations in the does during the first 2 years. By year three, there were no treatment differences likely due to the decline in the clover stands. Inclusion of WC or RC in TF swards resulted in similar forage yields as in N-fertilized TF and greater animal output when the percentage of clover was high \((\geq 30\%)\). Careful grazing management of TF-clover stands can reduce or eliminate the need for N fertilization. Reseeding clover every 2 to 3 years may be necessary to maintain optimal proportions of clover plants in the stand.

Keywords: meat goats, tall fescue, white clover, red clover, nitrogen cycling
**Introduction**

Incorporating legumes into grass pasture systems as a low-input nitrogen (N) source is not a new concept, but is regaining popularity. Grass-legume pasture mixtures were popular in the US in the early 1950’s before the price of synthetic N fertilizer decreased, stimulating increased prevalence of fertilized grass monocultures (Nelson and Burns, 2006). The comeback of forage legumes is due in part to the recent trends towards low-cost intensively managed grazing systems, as well as ecological farming in the US. Legumes have the ability to convert atmospheric N\textsubscript{2} into plant available N due to their synergistic relationship with soil rhizobia bacteria via the process of biological nitrogen fixation. The amount of fixed N that legumes contribute to a pasture ecosystem has been documented to range from 13 to 682 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} in mowed systems and 55 to 296 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} in grazed systems (Ledgard and Steel, 1992).

It is well-known that adding legumes to the grazing ruminant’s diet can improve live weight gain in sheep (*Ovis aries*; Fraser et al., 2004; Marley et al., 2005) and cattle (*Bos taurus*; Beck et al., 2012; Yarrow and Penning, 2001). Very little research has been published concerning goats (*Capra hircus*) grazing grass-legumes mixtures, with only one published account of legume effects on grazing meat goats (Turner et al., 2012). According to the last USDA Census of Agriculture, there were over two million meat goats in the United States with 8 of the 10 top producing states located in the south or southeast (USDA, 2012). Additional research is needed to help producers raise meat goats profitably on pastures.
Pasture mixtures containing legumes have noted advantages and challenges. Increased animal performance has been linked to greater forage nutritive values and increased voluntary dry matter intake (Rochon et al., 2004; Ulyatt, 1971). Unfortunately, the addition of white clover (*Trifolium repens* L.; WC) to grass stands has been reported to reduce annual forage production and consequently animal stocking rates when compared to fertilized grass monocultures (Beck et al., 2012; Yarrow and Penning, 2001). A decline is the proportion of clover in a mixed sward after 2 to 3 years of grazing has also been discussed at length (Vipond et al., 1997; Humphreys et al., 2009; Schaefer, 2014), although rotational versus continuous grazing has resulted in longer persistence (Cuttle, 1998).

The grazing behavior of sheep, cattle and goats heavily influences the clover content and persistence in a sward as well as subsequent animal performance. Rutter (2006) showed cattle and sheep showed a partial preference for 70% clover in their diet when offered a mixture. Sheep have a greater selectivity for clover than goats resulting in lower clover mass in the sward over time (Penning et al., 1996; del Pozo et al., 1996). Goats’ preference for browse species, weeds and grass leads to greater clover content in mixed swards (Clark et al., 1982).

While much research has been conducted on grass-WC mixtures in Australia, New Zealand and Europe, less has been reported in the United States. Tall Fescue (*Lolium arundinaceum* Shreb.; TF) is the dominant cool season perennial grass grown in the southeastern US. Because TF swards have open spaces between the upright, clump forming plants, it may be a better companion than ryegrass to WC (Hyslop et al., 2011). Two of the most popular legumes used in pasture mixtures include WC and red clover (*Trifolium*
pratense L.; RC). The WC is considered the best perennial forage legume for grazing because it can withstand repeated defoliation due to its stoloniferous growth. Conversely, RC is considered a biennial and used more often for hay or silage, because it does not persist well under grazing situations (Black et al., 2009). Polyphenol oxidase found in RC has been shown to reduce protein degradation rates in the rumen leading to increased ruminal bypass protein and N use efficiency of ruminants (Luscher et al., 2014). The objectives of this study were to 1) compare the effects of WC and RC to synthetic N in TF pasture DM (DM) yield and nutritive value 2) assess the changes in botanical composition of the swards over time due to rotationally grazing goats and mowing 3) determine how clovers and grazing animals impact grazing animal performance.

Materials and Methods

Experimental Site and Design

Plots were planted in September 2011 at the North Carolina State University Lake Wheeler Field Laboratories in Raleigh, NC. The soils were Appling fine sandy loam and Cecil gravelly sandy loam. The trial was replicated over 3 years (2012-2014). The experimental design was a split block with three field replicates and four main N treatments: TF with RC (TFRC), TF with WC (TFWC), TF fertilized with N (TFPOS) and TF with no additional N (TFNEG). Each plot measured 0.2 ha. Within each main treatment plot, the split blocks were grazed (G) and mowed (M) treatments at 2 sampling locations at either end of the plot. Grazing subplots measured 155 m² while mowed subplots measured 78 m².
Pasture Establishment

In August 2011, the existing forage at the research site (‘Max Q’ TF and ladino WC) was sprayed with Roundup® and then incorporated. ‘Max Q’ TF was planted with a no-till drill (Truax Company, New Hope, MN) at the rate of 19.6 kg ha\(^{-1}\) over the entire prepared field bed in September 2011. A mixture of ‘Cinnamon Plus’ and ‘Kenland Red’ RC was immediately overseeded at 10.7 kg ha\(^{-1}\) in September 2011 and again in February 2014. Cinnamon Plus was developed for better persistence under grazing pressure. ‘Will’ ladino WC was overseeded at 4.4 kg ha\(^{-1}\) in September 2011. This variety was developed at NCSU and is noted for persistence in hot climates. Because ladino WC had been growing in the field for one year previous to planting, it was assumed native rhizobia bacteria were in the soil. TFPOS plots were fertilized at 56 kg N ha\(^{-1}\) as ammonium sulfate in February and September each year. The plots were cut for hay once during each summer (June 2012 and August 2013). In March 2012 before the start of the trial, the grass only plots were sprayed with Weedmaster® to kill any volunteer clover. Plots were fertilized with phosphorus (58 kg P ha\(^{-1}\)) and potassium (149 kg K ha\(^{-1}\)) in September 2012 based on soil test results. No additional P or K was needed for the duration for the trial as per annual soil tests.

Animals and Grazing Management

The protocol for this study was approved by the Institutional Animal Use and Care Committee of North Carolina State University. Boer-cross and Kiko-cross lactating does with their twin kids were grazed during the springs (March – May) of 2012, 2013 and 2014. Does were assigned to one of 12 groups based on doe age, kid age and kid breed composition
in an effort to equalize all groups. The groups were then randomly assigned to replicate and treatment. Initial bodyweight of does were 48.5 kg ± 8.7 (2012), 51.2 kg ± 9.4 (2013) and 47.1 kg ± 8.0 (2014). The average age (d) and weight (kg) of the kids at the beginning of each trial was as follows: 2012- 17.8, 6.8; 2013 - 21.5, 8.2 and 2014 - 26.6, 6.7 kg, respectively. Grazing began in late March (3/18/12, 3/27/13 and 3/27/14) and lasted for 56 d. Stocking density varied per plot per year based on available forage with additional put and take animals used to equalize stocking density to approximately 500 kg forage ha⁻¹ per doe (forage allowance of 4.3 kg forage d⁻¹).

During fall 2012 (September – November), eight-month old wethers (n = 5 per plot with the exception of WC: n= 3.3/plot; initial BW 23.0 kg ± 6.7) were stratified by weight and then randomly assigned to treatment. They were only grazed for 39 d due to the lack of forage production. Additional put and take animals were used to equalize stocking density to approximately 450 kg forage ha⁻¹ per wether (forage allowance of 3.1 kg forage d⁻¹).

Animals were monitored for gastrointestinal parasite infections throughout the trial. Does were dewormed with levamisole hydrochloride (Prohibit®; 12 mg kg⁻¹) and moxidectin (Cydectin Drench for Sheep®; 0.3 mg kg⁻¹) at kidding several weeks prior to the start of the trial. At d 0 and d 28, a fecal egg count (FEC) was performed and a FAMACHA score was given to each doe (spring) and wether (fall) to assess gastrointestinal parasitic infection. Kids were also given a FAMACHA score at day 28. Animals that had FEC over 2,000 eggs per grm feces and/or a FAMACHA score of 4 or 5 while on trial were dewormed with either levamisole hydrochloride (Prohibit®; 12 mg kg⁻¹) or moxidectin (Cydectin Drench for Sheep®; 0.3 mg kg⁻¹).
Animals were moved to a fresh strip of forage (155 m$^2$) every two days (spring) or 2.5 days (fall) resulting in a grazing cycle length of 23 days (spring) and 29 days (fall). Animals were given a mineral supplement (Cattleman’s Pride Weathershed 2:1 Beef Mineral; Southern States Cooperative, Inc., Richmond, VA) three times a week at the rate of 28 g hd$^{-1}$ d$^{-1}$. Forage in the mowed subplots was harvested with a walk behind sickle bar mower (Jari Mowers, Mankato, MN) at the same time animals were grazing the corresponding G subplots. Forage was mowed to an average height of 7.5 cm in an effort to match post-grazing height. Cut forage was then removed from the plots to prevent nutrient recycling.

**Forage Sampling and Analysis**

Estimates of forage mass for treatment effects were determined in the G and M subplots (2 G and 2 M per plot) for each grazing cycle using a 0.25m$^2$ falling plate meter three days before grazing/mowing. Ten height measurements were taken in the G subplots and 5 in the M subplots. Six (0.25m$^2$) quadrats were clipped to 5.0 cm for each treatment and each harvest type representing the range of forage mass present. Two sites represented low forage mass, two sites represented medium forage mass and two sites represented high forage mass. Clipped forage was dried at 60° C for 48 h and weighed hot to determine DM yield (kg ha$^{-1}$). The equation developed from the linear regression of plate meter height against DM yield was used to predict available forage mass before grazing. These equations were used to estimate forage mass for determination of stocking density every 4 days.
Calibrations were repeated for each grazing cycle. Post-harvest forage mass was determined using the same procedure.

Forage was sampled for determination of nutritive value in the G and M subplots before each grazing/mowing event. Forage was clipped 5 cm from ground level at 12 randomly selected sites within G subplots and 6 sites from M subplots and then hand separated into four fractions consisting of green TF, brown/dead TF, clover and ‘other’ species. Fractions were then dried at 60° C for 48 h and weighed hot to determine the percent of each fraction in the sward on a DM basis.

Fractions were ground through a 1 mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and stored in whirl-pak bags (NASCO, Modesto, CA) at room temperature until analyzed. Dry matter, Kjeldahl nitrogen, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by AOAC procedures (1999). The NDF, ADF and lignin were determined sequentially according to Van Soest et al. (1991) using the ANKOM™ Fiber Analyzer (Ankom Corp., Fairpark, NY). Cellulose was calculated as the difference between ADF and the 72% sulfuric acid residue. Total N was determined by auto analyzer (Technicon Industrial Systems, Tarrytown, NY).

Animal Data Collection and Analysis

Animals were weighed beginning at 0800 on d 0, 28 and 56. Average daily gain (ADG; g d⁻¹) and gain per hectare (kg gain ha⁻¹) were calculated. Animal BW gain per hectare was calculated using the following equation: [ADG (kg d⁻¹)*animals (hd)*length of grazing period (d)]/total area grazed (ha).
Blood samples were collected via jugular venipuncture at d 0, 28 and 56 to determine serum urea nitrogen (SUN) levels. Blood was collected in vacuum tubes without additive (Becton Dickinson, Franklin Lakes, NJ) and put on ice until they were centrifuged approximately 6 h later at 1,900 g for 30 min. After the serum was extracted, the samples were frozen at -15° C until analyzed by colorimetry using an auto analyzer (Technicon Industrial Systems, Tarrytown, NY).

Feces were digitally collected at d 0, 28 and 56 and divided into 2 portions for determination of gastrointestinal parasite infection and fecal N concentration. Individual animal samples were analyzed for parasites using a Modified McMasters Technique (Paracount-EPG™, 1984). Samples for fecal N were composited by plot, dried at 40° C in a forced air oven and ground through a 1 mm Wiley Mill screen (Thomas Scientific, Swedesboro, NJ). Samples were stored at room temperature in whirl-pak bags (NASCO, Modesto, CA) until they were analyzed. Samples were digested using the Kjeldahl N method (AOAC, 1999) and total N was determined by colorimetry with an auto-analyzer (Technicon Industrial Systems, Tarrytown, NJ). Urine samples were collected from does on d 28 and 56 for determination of N concentrations. Immediately after collection, samples were placed on ice and then frozen at -15° C within 6 h. Samples were analyzed similarly to fecal N.

Statistical Analysis

Analysis of variance using the PROC GLM procedure of SAS, version 9.3 (SAS Institute, 2008) was used to test the effects of N treatment, harvest type, year, N treatment x harvest type, N treatment x year and harvest type x year for all forage characteristics. PROC GLM
was also used to test the effects of N treatment on animal performance. The LSMEANS option was used to generate individual treatment means. Treatment means were further separated using the Fisher’s least significant difference test (Steel et al., 1997). Significance was declared at $P < 0.05$ and tendencies of $P < 0.10$ are reported.

**Results and Discussion**

*Weather*

In 2012 and 2013, precipitation during the growing season of March – November was 86% of the 30 year average, whereas in 2014 more precipitation fell than normal (Figure 2.1). The mean monthly air temperatures in 2012 and 2013 were consistent with the 30 year average (Figure 2.2). The first three months of 2014 saw lower than normal mean air and soil temperatures. This may have negatively impacted the overall forage DM yield and clover growth during the spring grazing months of March through May.

*Botanical Composition*

Tables 2.1 and 2.2 summarize the botanical composition of the swards for spring and fall by year as a treatment by year interaction was observed ($P < 0.001$ and $P < 0.05$, respectively). The proportion of TF DM in the plots fertilized with synthetic N stayed consistently above 90%. In the fall 2012, the proportion of TF in all plots declined from the spring and only ranged from 32.8% - 66.9% due to a large invasion of summer annual weeds (Table 2.2). The large amount of dead material in spring 2014 was likely a result of the lack of grazing during fall 2013 even though the field was mowed once in late winter.
There was a treatment by year interaction \((P < 0.05)\) of the proportion of clover in the TFWC and TFRC swards (Figure 2.3). During the spring, the proportion of white clover in the sward was similar in 2012 and 2013 at 40.4 and 43.2\%, respectively but declined to 18.7\% in 2014. The proportion of RC DM declined from 30.0\% in 2012 to 11.5\% in 2013 and 2.6\% in 2014 resulting in less RC DM than WC during the spring of 2013 and 2014 \((P < 0.05)\). The decline in WC after 2 to 3 y is consistent with other studies that have reported difficulty maintaining clover in grazed mixed swards over time (Vipond et al., 1997; Humphreys et al., 2009; Schaefer, 2014). Legumes capitalize on low soil N environments. Animals grazing forages high in N will excrete urine and feces high in N leading to increased soil N, thus giving an advantage to grass growth in a mixed sward (Schwinning and Parsons, 1996; Phelan, 2015).

The clover component of the sward was less in fall compared to spring for both clover species \((P < 0.01)\) with TFWC producing greater clover DM than TFRC \((P < 0.05)\; \text{Figure 2.3}\). The stolon growth of WC is slow in spring, peaks during summer and steadily declines through fall and winter as old plants die and are not replaced (Black et al, 2009).

Mowed plots had a greater proportion of clover than the grazed plots during spring 2012 and 2013 (39.8 vs 30.7\% and 35.3 vs 19.4\%, respectively; \(P < 0.05\)). Clover production is generally greater in mowed swards versus grazed, because mowing depletes soil N, thus increasing BNF and competitiveness of the clover against the grass forage (Frame and Newbould, 1986; Nyfeler et al., 2011). The proportion of clover in mowed swards has been reported to be double the proportion of grazed treatments (Eriksen and Hogh-Jensen, 1998).
Forage Dry Matter Yield

During the spring and fall grazing seasons, differences in forage DM yields were found between treatments and between years, but there were no interactions (Figure 2.4 a & b). During spring 2012, the forage DM yield upon entry into the paddocks for TFWC and TFRC were similar to TFPOS. In spring 2013, TFPOS was equal to TFWC but greater than TFRC and TFNEG \((P < 0.01)\). By 2014, the DM yield of TFPOS was greater than all other treatments \((P < 0.001)\). During both fall 2012 and 2013, TFWC had less forage DM yield than TFPOS and TFRC \((P < 0.05)\). Due to the severe decline of RC in the fall, the TFRC plots had a large weed infestation which may have led to the increased forage DM yield.

Available forage DM declined each year from 2012 through 2014 \((P < 0.05)\) across all treatments. The DM yield of grazed plots was greater than of mowed plots only in spring 2013 and fall 2012.

The annual DM yield (DM/grazing cycle/season * number of grazing cycles) of both the TFWC and TFRC treatments was 84% of the fertilized TF treatment \((7.57, 7.56 \text{ and } 8.98 \text{ t ha}^{-1}, \text{respectively})\). These results are in agreement with the majority of the literature reporting grass-legume swards producing 77 to 89% of the DM yield of N fertilized ryegrass or orchardgrass pastures (Curll et al., 1985; Orr et al., 1990; Vipond et al., 1997; Ledgard et al. 2001; Papadopoulos et al., 2001). On the contrary, TF over-seeded with either RC or WC produced similar annual yields to TF fertilized with 180 or 190 kg N ha\(^{-1}\) (Vines et al., 2006; Schaefer et al., 2014).
Crude Protein Concentration of Forages

The CP analysis of the forage components showed a treatment by year interaction ($P < 0.001$) for both fescue and clover during the spring (Table 2.3). The CP of the TF in TFWC plots was similar to that TFPOS in 2012 (15.5 vs 15.1%, respectively), greater than of TFPOS in 2013 (18.2 vs 16.5%, respectively) but lower than of TFPOS in 2014 (18.5 vs 19.9%, respectively; $P < 0.001$). The TF CP values in TFRC plots were intermediate, while TFNEG had the lowest CP concentrations in 2012 and 2014 ($P < 0.001$). Clover in TFWC had greater CP concentration than clover in TFRC in all three years (avg 28.9 vs 26.0%, respectively; $P < 0.01$). Ulyatt et al. (1988) documented WC monocultures of 22.9% CP. The CP of grazed TF was greater than mowed TF in 2013 only (15.9 vs 15.1%, respectively; $P < 0.05$).

During fall, the treatment x year interaction for TF CP concentration was not significant ($P = 0.08$), but data are presented for each year separately because of the difference in management (Table 2.4). Fescue in TFPOS swards had similar CP to fescue in TFWC in 2012 and 2013 and TFRC in 2012 ($P < 0.01$). No differences were seen in harvest type. The CP levels of grass have been shown to be greater in the fall than in the spring as a result of increased soil N mineralization and availability (INRA, 2007). The CP concentration of WC in fall of 2012 and 2013 was 27.2 and 28.5%, respectively. There was no RC growth to sample. Clover CP concentration in grazed TFWC was greater than mowed TFWC (27.6 vs 26.8% CP, respectively; $P < 0.05$).
Spring sward CP values are presented in Table 2.5, and a treatment by year interaction was observed ($P < 0.001$). In spring 2012, TFWC treatments had the greatest sward CP, followed by TFRC, TFPOS and TFNEG (19.9, 17.6, 15.1, 12.4% CP, respectively; $P < 0.001$). The TFWC plots had greater sward CP than TFPOS in 2013 but the two were similar in 2014 likely due to the decrease in proportion of WC. These forage diets meet the CP requirement for lactating goats which ranges from 11 to 14% based on milk production (NRC, 1981). These results also agree with Schaefer et al. (2014) who reported greater CP values of TF-WC swards compared to fertilized TF monocultures (17.2 vs 15.7% CP, respectively). Yarrow and Penning (2001) found that as the proportion of WC in perennial ryegrass pastures increased, the N concentration of the sward also increased.

By fall, the TFWC treatment had lost its advantage in CP concentration over TFPOS coinciding with the lower percentage of WC DM. In fall 2012, TFWC and TFPOS had similar sward CP (18.0 and 16.4% CP, respectively) and were greater ($P < 0.01$) than TFNEG and TFRC (13.4 and 12.6% CP, respectively). By fall 2013, CP of TFPOS was greater than TFWC which was in turn greater than that of TFRC and TFNEG (18.9, 15.9, 12.9 and 12.0 % CP, respectively; $P < 0.01$).

The TDN: CP ratio of the swards varied between treatments TFWC swards having the lowest values every year ($P < 0.01$; Table 2.5). The TDN: CP requirement of lactating goats ranges from 4.6 to 5.4 (NRC, 1981). All treatments except TFNEG fell below this level indicating low energy values relative to excess CP in forage most likely resulting in greater N excretion (Turner et al., 2012).
Fiber Analysis

The NDF and ADF values of the swards were positively impacted by the inclusion of clovers. There was a treatment by year interaction ($P < 0.001$), but for each of three years, TFWC and TFRC had lower NDF values than TFPOS ($P < 0.001$). Inclusion of WC also resulted in lower sward ADF values than the grass monocultures each of the three years ($P < 0.01$). The ADF value of TFRC swards was only lower than the TF-only swards in 2012 when the proportion of RC was high. These results agree with earlier studies indicating legumes typically have lower fiber concentrations and higher digestibility than grasses (Jamot and Grenet, 1991; Sturludottir et al., 2014).

Animal Performance - Lactating does and kids

There were no treatment differences for kid ADG during any of the spring grazing seasons (avg = 135.3 g/d; Table 2.6). These gains are consistent with other studies that have measured the performance of kids suckling does that were grazing grass or grass-forb pastures: 116 g d$^{-1}$ (Goetsch et al., 2014), 129.5 g d$^{-1}$ (Browning et al., 2011) and 132 g d$^{-1}$ (Goetsch et al., 2007). While the lactating does lost weight during all three years, no differences were observed between treatments in any of the years. Nursing does have been shown to mobilize their own body tissues to raise their kids (Goetsch et al., 2007). Although Turner et al. (2012) documented an increase in ADG (34g d$^{-1}$) for growing, weaned goats grazing grass-RC over those grazing orchardgrass (Dactylis glomerata L.) monocultures, Orr (1990) observed no difference in performance of nursing lambs managed on either perennial ryegrass-clover swards or fertilized ryegrass swards. Likewise, Butler et al. (2012) found
similar ADG for steers grazing annual legume-rye/ryegrass mixture to fertilized annual rye/ryegrass pastures.

Increased milk and meat production of animals on diets containing legumes is linked in part to greater voluntary dry matter intake (DMI) when animals have *ad libitum* access to diets (Rochon et al., 2004; Dewhurst et al., 2009; Steinshamn, 2010). In the current study an attempt was made to keep the amount of forage offered per doe (4.3 kg forage d\(^{-1}\)) consistent between treatments by estimating forage biomass every other move (every 4 d) and adjusting put and take animals accordingly. Although it is impossible to calculate the exact DMI of each doe and kid, after grazing DM mass measurements were taken to help estimate forage disappearance and DMI of each doe-kids trio. For each of the three years, the estimated DMI for TFWC was similar to TFPOS (avg = 2.04 kg DM d\(^{-1}\) per trio), while TFRC was similar to TFPOS in 2012 and 2013. DMI for TFNEG was lower than the other treatments in 2012 and 2013 at 1.2 kg DM d\(^{-1}\) per trio. Harris et al. (1998) did not observe an increase in DMI or milk production when animals were offered a restricted diet of grass-WC mixture.

A treatment by year interaction (*P* < 0.001) was observed for the kid gain per hectare (Table 2.6). In 2012, TFRC was greater than TFPOS (352 vs 289 kg ha\(^{-1}\); *P* < .001) while TFWC was similar to both at 330 kg ha\(^{-1}\). In 2013 and 2014, TFWC was greater (*P* < .001) than all other treatments. TFRC was similar to TFPOS in year 2013 but lower than TFPOS in year 2014. The TFNEG treatment had the lowest animal output each year (*P* < 0.001). Turner et al. (2012) observed animal outputs of 94 to 202 kg ha\(^{-1}\) for goats grazing orchardgrass or orchardgrass-red RC, although there was no difference between treatments.
Schaefer (2014) reported greater BW gain ha\(^{-1}\) of steers when the WC DM content of sward was greater than 45\% versus those grazing grass fertilized at 190 kg N ha\(^{-1}\). This advantage in animal output decreases as clover becomes a smaller proportion of the sward or grass is fertilized with greater than 200 kg N ha\(^{-1}\) annually (Burns and Standaert, 1985; Vipond et al., 1997; Schaefer et al., 2014).

There was a treatment by year interaction for doe stocking density \((P < 0.05)\). The stocking density of does per hectare was similar for TFWC and TFPOS for all three years (Table 2.6). The stocking density for TFNEG was lower \((P < 0.01)\) than other treatments all three years. During 2012, TFRC was similar to TFWC and TFPOS, but lower than these treatments in 2013 and 2014, due to its lower forage DM yield. The fact that TFPOS and TFWC were similar is in contrast to previous work documenting the stocking rate of ewes and twin lambs grazing perennial ryegrass-WC at 63\%, 77\% and 82\% of ryegrass swards fertilized with 160, 420 and 190 kg N/ha, respectively (Orr et al., 1990; Vipond et al., 1993; 1997). Perhaps the difference was the amount of N fertilizer used as in the current study was only 112 kg N ha\(^{-1}\) yr\(^{-1}\) was applied and the resulting TFPOS yield was the same as TFWC in spring.

**Blood, Fecal and Urine Nitrogen**

There was a treatment by year interaction for the SUN measurements \((P < 0.001)\). In 2012, the TFWC and TFRC treatments were similar to each other and greater than both the TFPOS and TFNEG treatments on day 28 (27.4, 25.4, 18.9 and 15.0 mg dl\(^{-1}\), respectively; Figure 2.5 a; \(P < 0.01\)) and day 56 (28.2, 26.0, 18.0 and 9.9 mg dL\(^{-1}\), respectively; \(P <\)
In 2013, TFWC was greater than TFRC, TFPOS and TFNEG on d 28 (27.0, 20.9, 17.7 and 17.1 mg dL\(^{-1}\), respectively; Figure 2.5 b; \(P < 0.05\)) and d 56 (25.3, 18.3, 16.5 and 15.7 mg dL\(^{-1}\), respectively; \(P < 0.05\)). By year three, there were no treatment differences on any samples dates. Sahlu et al. (1993) reported BUN measurements of 8.3, 22.0 and 33.3 mg dL\(^{-1}\) for diets containing 8.5, 13.9 and 20.3% CP, which are similar to the current experiment. BUN levels have been shown to increase as the dietary protein levels fed to growing goats increases (Sahlu et al., 1993; Turner et al., 2005). High BUN levels are correlated with high ruminal ammonia caused by low energy: nitrogen ratio in the rumen (Hammond et al., 1994).

There was a treatment by year interaction for fecal N of lactating does (\(P < 0.05\)). In 2012, TFWC was greater than TFRC which was in turn greater than TFPOS and TFNEG on d 28 (3.1, 2.8, 2.4 and 2.5 %N, respectively; \(P < 0.05\)). In 2013, TFWC was again greater than TFRC, TFPOS and TFNEG on d 28 (3.1, 2.7, 2.6 and 2.4 %N, respectively; \(P < 0.01\)). At d 56, TFWC was similar to TFPOS but greater than TFRC and TFNEG (\(P < 0.05\)). There were no treatment differences during 2014 on any sample date. Animals grazing diets high in N have been shown to have greater fecal and urine N concentration (Wofford, 1985; Dewhurst et al., 2009). Orr et al. (1995) reported fecal N concentrations from sheep grazing clover monocultures (3.9% N), grass-clover mixtures (3.10% N), grass fertilized with 420 kg N/ha (3.5% N) and unfertilized grass (3.0% N).

Partly due to low number of samples and a high variability among animals, there were no differences in urine N at d 28 or 56 (\(P = 0.22\) and 0.28, respectively). Mean values on d 28 for TFWC, TFRC, TFPOS, and TFNEG were 0.104, 0.103, 0.082 and 0.073 mg N mL\(^{-1}\) urine, respectively.
Fall 2012 Wethers

There was no difference in ADG of wethers grazing during fall 2012 (avg = 71.2 g/d; \( P = 0.20 \)) The stocking density for the TFPOS and TFRC wethers grazing during fall 2012 was greater than TFWC due to the greater DM yield (TFPOS: 32.8; TFRC: 30.7; TFNEG: 29.2; TFWC: 25.5 animals ha\(^{-1}\); \( P < 0.05 \)). The BW gain per hectare during fall 2012 was similar between TFRC, TFPOS, TFWC and lower in TFNEG (91.0, 82, 81, 60 kg ha\(^{-1}\), respectively; \( P < 0.01 \). The only difference in SUN values was measured on d 56 as TFWC was greater than TFRC, TFNEG and TFPOS (19.8, 17.4, 17.4 and 17.0 mg dL\(^{-1}\); \( P < 0.01 \). There were no differences in fecal N or urine N concentrations. These results are not surprising considering the large percentage of summer annual weeds and lower percentage of clover in the stands.

Conclusion

The current research compared the effects of adding WC, RC or applying N fertilization to TF pastures. The proportion of the clover DM in the stand declined from year 1 to year 3 and impacted forage yield and chemical composition. WC was able to persist longer than RC under repeated defoliation. Overseeding WC into the TF increased the nutritive value (CP, NDF and ADF) of the sward. Individual animal performance was not affected by addition of the clovers, possibly because DMI was similar between clover based and fertilized treatments, but high levels of clover in the sward did increase animal gain per hectare. Does grazing TFWC had greater N intake and N excretion. During the spring grazing seasons, the forage DM yield of the clover treatments was similar to N fertilized
treatment when the swards contained at least 30% clover DM. Forage production in the WC treatment was less during the fall growing season. Mowing pasture swards led to an increase in clover percentage in two out of three years. Overall, adding WC to TF pastures in rotational grazing systems for goats proved productive during the spring season and can reduce the need for N fertilization.

**Implications**

This research has shown that when the proportion of RC or WC is between 20 and 30% of a TF sward, the pasture system can produce similar amounts of forage and animal growth compared to application of synthetic N fertilizer. The mixed TF-clover plots in the current study performed well in the spring but were less productive during the fall. RC and WC are highly nutritious and palatable legumes that will produce goat kid gains comparable to those found in other published studies. Sustainable, organic and/or grass-fed producers can use these legumes as a low input N source for grazing livestock and potentially increase profitability. Mixed TF-clover pastures should be managed using strip grazing or rotational grazing to ensure the TF growth does not get too tall and shade out the clovers, and to prevent preferential grazing of clover plants. Reseeding cool season grass pastures with clovers every 2 to 3 years may be needed to maintain the optimal proportion.
REFERENCES


Paracount-EPG . 1984. Veterinary Quantitative Fecal Analysis Kit. Professional modified McMaster egg counting technique for calculating parasite egg per gram (EPG) of feces samples. Olympic Equine Products. Issaquah, WA


Table 2.1. Botanical composition (% DM) of tall fescue pastures with varying N sources from late March through mid-May 2012, 2013 and 2014. (Treatment by year interaction $P < 0.001$)

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>TFRC</th>
<th>TFWC</th>
<th>TFPOS</th>
<th>TFNEG</th>
<th>SEM</th>
<th>$P$-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012 - Fescue</td>
<td>66.8b</td>
<td>52.2c</td>
<td>99.7a</td>
<td>99.7a</td>
<td>3.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Clover</td>
<td>30.0a</td>
<td>40.4a</td>
<td>NA</td>
<td>NA</td>
<td>4.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Other**</td>
<td>3.9b</td>
<td>8.3a</td>
<td>0.18c</td>
<td>0.33c</td>
<td>0.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Dead</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013 - Fescue</td>
<td>65.2b</td>
<td>51.7b</td>
<td>91.5a</td>
<td>84.2a</td>
<td>3.9</td>
<td>&lt; 0.01</td>
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<tr>
<td>Clover</td>
<td>11.5b</td>
<td>43.2a</td>
<td>NA</td>
<td>NA</td>
<td>5.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Other**</td>
<td>8.4b</td>
<td>3.4c</td>
<td>8.1b</td>
<td>14.8a</td>
<td>0.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Dead</td>
<td>17.7b</td>
<td>2.6a</td>
<td>0.4a</td>
<td>0.4a</td>
<td>3.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2014 - Fescue</td>
<td>77.1b</td>
<td>68.9c</td>
<td>91.6a</td>
<td>81.8b</td>
<td>1.8</td>
<td>&lt; 0.01</td>
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<tr>
<td>Clover</td>
<td>2.6b</td>
<td>18.7a</td>
<td>NA</td>
<td>NA</td>
<td>2.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Other**</td>
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<td>2.3a</td>
<td>0a</td>
<td>1.1a</td>
<td>1.1</td>
<td>0.08</td>
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<tr>
<td>Dead</td>
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<td>9.9bc</td>
<td>8.11c</td>
<td>17.1a</td>
<td>1.1</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

a-c means with differing superscript differ within rows
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)
* $P$-value of treatment effect
** “other” forage species include mainly *Stellaria media*, *Lamium amplexicaule* L. and *Lolium perenne*
Table 2.2. Botanical composition (% DM) of tall fescue pastures with varying N sources during fall (September – November) 2012 and 2013. (Treatment by year interaction $P < 0.05$)

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>TFRC</th>
<th>TFWC</th>
<th>TFPOS</th>
<th>TFNEG</th>
<th>SEM</th>
<th>$P$-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012 - Fescue</td>
<td>32.8</td>
<td>40.2</td>
<td>66.9</td>
<td>52.2</td>
<td>7.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Clover</td>
<td>0.6b</td>
<td>18.4a</td>
<td>NA</td>
<td>NA</td>
<td>2.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Other**</td>
<td>68.7a</td>
<td>38.9b</td>
<td>34.2b</td>
<td>50.4ab</td>
<td>6.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Dead</td>
<td>3.1</td>
<td>3.1</td>
<td>7.5</td>
<td>4.9</td>
<td>1.00</td>
<td>0.07</td>
</tr>
<tr>
<td>2013 - Fescue</td>
<td>63.5c</td>
<td>77.2b</td>
<td>88.8a</td>
<td>63.7c</td>
<td>3.12</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Clover</td>
<td>0.5b</td>
<td>2.5a</td>
<td>NA</td>
<td>NA</td>
<td>0.21</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Other**</td>
<td>10.7bc</td>
<td>16.3ab</td>
<td>9.4c</td>
<td>17.8a</td>
<td>1.79</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Dead</td>
<td>25.3a</td>
<td>4.0bc</td>
<td>1.8c</td>
<td>18.4ab</td>
<td>4.67</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

a-c means with differing superscript differ within rows
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)
* $P$-value of treatment effect
** “other” forage species include mainly Digitaria ischaemum, Digitaria sanguinalis, and Setaria pumila
Table 2.3. Crude protein (% DM) of fescue and clover components in pasture swards with varying N sources during spring 2012, 2013 and 2014. (Treatment x year interaction $P < 0.001$)

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Forage, % CP</th>
<th>TFRC</th>
<th>TFWC</th>
<th>TFPOS</th>
<th>TFNEG</th>
<th>SEM</th>
<th>$P$-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fescue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>14.2$^b$</td>
<td>15.5$^a$</td>
<td>15.1$^{ab}$</td>
<td>12.4$^c$</td>
<td>0.27</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>14.0$^c$</td>
<td>18.2$^a$</td>
<td>16.5$^b$</td>
<td>13.5$^c$</td>
<td>0.34</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>16.2$^c$</td>
<td>18.5$^b$</td>
<td>19.9$^a$</td>
<td>15.6$^d$</td>
<td>0.16</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Clover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>25.8$^b$</td>
<td>27.1$^a$</td>
<td>NA</td>
<td>NA</td>
<td>0.06</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>25.9$^b$</td>
<td>29.4$^a$</td>
<td>NA</td>
<td>NA</td>
<td>0.09</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>26.4$^b$</td>
<td>30.1$^a$</td>
<td>NA</td>
<td>NA</td>
<td>0.19</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

a-d means with differing superscript differ within rows
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)
* $P$-value of treatment effect
Table 2.4. Crude protein (CP; % DM) of fescue and clover components in pasture swards with varying N sources during fall (September – November) 2012 and 2013.

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Forage, % CP</th>
<th>TFRC</th>
<th>TFWC</th>
<th>TFPOS</th>
<th>TFNEG</th>
<th>SEM</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fescue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>20.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>17.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Clover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>NA</td>
<td>27.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2013</td>
<td>NA</td>
<td>28.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

a-c means with differing superscript differ within rows
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)
* P-value of treatment effect
Table 2.5. Chemical composition (% DM) of tall fescue and tall fescue-clover mixtures in pastures within three spring (March – May) grazing seasons (treatment x year interaction $P < 0.001$)

<table>
<thead>
<tr>
<th>Year</th>
<th>Forage†</th>
<th>CP</th>
<th>NDF</th>
<th>ADF</th>
<th>TDN:CP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(DM)</td>
<td>(DM)</td>
<td>(DM)</td>
<td>(DM)</td>
</tr>
<tr>
<td>2012</td>
<td>TFRC</td>
<td>17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFWC</td>
<td>19.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>15.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>12.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>56.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SEM</td>
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<td>1.17</td>
<td>0.57</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2013</td>
<td>TFRC</td>
<td>14.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>62.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFWC</td>
<td>22.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>16.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>13.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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<td>SEM</td>
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<td>1.08</td>
<td>0.48</td>
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<td>P-value</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2014</td>
<td>TFRC</td>
<td>15.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>TFWC</td>
<td>20.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>19.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>15.1c</td>
<td>61.5b</td>
<td>30.3a</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>SEM</td>
<td>0.20</td>
<td>0.56</td>
<td>0.28</td>
<td>0.05</td>
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<tr>
<td></td>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; TDN = Total Digestible Nutrients
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)

a-d means with differing superscript differ within columns
Table 2.6. Average daily gain (ADG), kid gain per hectare and stocking density of meat does and their nursing kids strip-grazed on tall fescue with varying N source from late March through mid-May 2012, 2013 and 2014

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment†</th>
<th>TFRC</th>
<th>TFWC</th>
<th>TFPOS</th>
<th>TFNEG</th>
<th>SEM</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kid ADG, g d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144.9</td>
<td>144.9</td>
<td>119.4</td>
<td>132.0</td>
<td>8.58</td>
<td>0.19</td>
</tr>
<tr>
<td>Doe ADG, g d⁻¹</td>
<td></td>
<td>-17.3</td>
<td>-49.4</td>
<td>-22.1</td>
<td>-9.2</td>
<td>13.62</td>
<td>0.27</td>
</tr>
<tr>
<td>Kid gain per hectare, kg ha⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td>352</td>
<td>330</td>
<td>289</td>
<td>200</td>
<td>13.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td>253</td>
<td>302</td>
<td>263</td>
<td>140</td>
<td>6.9</td>
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</tr>
<tr>
<td>2014</td>
<td></td>
<td>166</td>
<td>271</td>
<td>238</td>
<td>115</td>
<td>8.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Stocking density, does ha⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td>21.3</td>
<td>22.8</td>
<td>21.7</td>
<td>13.0</td>
<td>1.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td>16.4</td>
<td>20.8</td>
<td>23.4</td>
<td>11.9</td>
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<td>&lt; 0.01</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td>10.3</td>
<td>16.1</td>
<td>17.8</td>
<td>7.3</td>
<td>0.5</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

a-d means with differing superscript differ within rows
† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized tall fescue); TFNEG = negative control (tall fescue no N)
* P-value of treatment effect
Figure 2.1. Mean monthly precipitation (mm) in Raleigh, NC during 2012-2014 compared to the 30 year average (1981-2010).
Figure 2.2. Mean monthly air temperature (°C) in Raleigh, NC during 2012-2014 compared to the 30 year average (1981-2010).
Figure 2.3. Percentage of clover dry matter (DM) in grazed (G) or mowed (M) tall fescue-red clover (RC) and tall fescue-white clover (WC) pasture swards during spring 2012, 2013 and 2014 and fall 2012 and 2013

(Spring vs Fall 2012 and 2013; \( P < 0.01 \))

* WC vs RC; \( P < 0.05 \)
**Figure 2.4.** Available forage dry matter (kg DM ha\(^{-1}\)) upon entry into paddocks of tall fescue plots with either white clover, red clover, nitrogen fertilization (positive) or no additional N (negative) during spring 2012-2014 (a) and fall 2012 and 2013 (b) grazing seasons.

* P < 0.05; ** P < 0.01; *** P < 0.001
Figure 2.5 Serum urea nitrogen (mg dL$^{-1}$) of lactating does grazing tall fescue plots with either white clover, red clover, nitrogen fertilization (positive) or no additional N (negative) during spring 2012 (a), 2013 (b) and 2014 (c). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
CHAPTER 3

Impact of grazing and clovers on biological nitrogen fixation and soil nitrogen status in tall fescue pasture ecosystems


Abstract

Grazing studies were conducted in spring 2012, 2013 and 2014 and fall 2012 to evaluate the effects of meat goats and legumes on nitrogen (N) cycling in tall fescue (Lolium arundinaceum Shreb.; TF) pasture ecosystems. Objectives were to measure the effects of N source (legumes versus N fertilizer) and grazing/excreta on biological nitrogen fixation, forage N yield and soil N status. The legumes used were white clover (Trifolium repens, L.; WC) and red clover (Trifolium pratense L.; RC). The experiment was a split block design with 4 main N treatments: TF mixed with RC (TFRC), TF mixed with WC (TFWC), TF fertilized with 112 kg N ha$^{-1}$ (TFPOS) and TF with no additional N (TFNEG). Within each main treatment plot, there were grazed (G) and mowed (M) subplots. $^{15}$N Natural Abundance technique was used to calculate %N derived from the atmosphere (%Ndfa) of clovers. Soil samples were taken before and after grazing/mowing and weekly thereafter and analyzed for nitrate-N and ammonium-N. The average %Ndfa during spring was 82% for the RC and WC and 42% during the fall for the WC. This decline in %Ndfa was correlated to an increase in soil nitrate-N levels ($P < 0.05$). The WC fixed a total of 121 and 76 kg N ha$^{-1}$ in 2012 and 2013, respectively. The RC fixed 62 kg N ha$^{-1}$ in 2012 but only 15 kg N ha$^{-1}$ in 2013, due to a significant decrease in clover DM proportion in the sward. The TFWC had
greater soil NH$_4$-N compared to the fescue-only treatments in two of the three years and greater soil NO$_3$-N in each of the three years. Grazed treatments had greater inorganic soil N than mowed plots. Swards containing clover had lower C:N ratio which likely led to greater N mineralization and greater soil N levels even in the mowed treatments. The TFWC produced the greatest amount of forage N during spring ($P < 0.01$), but TFPOS produced the greatest amount of forage N during the fall ($P < 0.01$). Adding grazing animals and/or clovers to TF pastures increased forage and soil N cycling but lead to increased soil NO$_3$-N levels which negatively impacted biological nitrogen fixation.

Keywords: Biological nitrogen fixation, clover, tall fescue, inorganic soil nitrogen
**Introduction**

While graziers may include forage legumes into pasture systems to achieve greater forage nitrogen (N) concentration and increased animal weight gain, legumes are also used as a low input alternative to N fertilizer. Legumes have the ability to convert atmospheric N\textsubscript{2} into plant available N due to their synergistic relationship with soil rhizobia bacteria via the process of biological nitrogen fixation (BNF). Rhizobia bacteria (supplied either through seed inoculation or native soil populations) infect legume roots to form nodules where N\textsubscript{2} fixation occurs. The amount of fixed N that legumes contribute to a pasture ecosystem has been documented to range from 13 to 682 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} in mowed systems and 55 to 296 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} in grazed systems (Ledgard and Steel, 1992) possibly reducing or eliminating the need for synthetic N in grass-legume mixed swards (Elgersma and Hassink, 1997).

Although a multitude of ecosystem level benefits may exist for including legumes in pastures, quantifying the contribution of BNF, and therefore management, is challenging. For grass-legume mixtures to have an advantage in dry matter (DM) yield over grass-only pastures, the biologically fixed N must be transferred to non-legume companion plants. The above ground pathway involves animals grazing and recycling N back onto the pasture through excreta. Grazing ruminants only convert 5 to 25% of their dietary N into animal proteins and excrete the remainder back onto the pasture through urine and feces with urine accounting for 75% of the total N excreted (Ulyatt et al., 1988; Whitehead, 1995). Ledgard (1991) estimated 60 kg of white clover (*Trifolium repens* L.) derived N ha\textsuperscript{-1} yr\textsuperscript{-1} was transferred to perennial ryegrass (*Lolium perenne* L.) in a mixed pasture through cattle excreta. Unfortunately, an increase in inorganic soil N caused by excreta or legume plant
mulch usually decreases BNF and legume proportion in the sward as the grass out competes
the clover for resources (Meener et al., 2003; Hatch et al., 2007) This can lead to varying and
unpredictable amounts of biologically fixed N through the year and between years.

Fixed N is also transferred below ground through root exudates and the
decomposition of the legume’s shoots, roots and nodules. Greater mineralization of legume
plant material is correlated with lower C:N ratios and lignin:N ratios compared to grasses
(Thomas and Asakawa, 1993; Ranells and Wagger, 1996). The amount of N transferred
through root and nodule composition can range from 2 to 26% of BNF in mixed pastures
(Ledgard and Steele, 1992)

Excessive N in agricultural systems can have a negative impact on N losses to the
environment. Often, the excessive application of fertilizer N in cropland and pastures is
blamed for polluting water sources (Thornburn, et al. 2013). Although forage legumes add
nitrogen to a system, their negative feedback system may limit BNF when soil N levels are
already high and naturally mediate N inputs and losses. When animals ingest a diet high in
N, their urine will be high in urea N which can be lost as ammonia through volatilization or
mineralized into inorganic soil N (ammonium or nitrate) for use by plants. A single
urination on average can deliver 200 to 800 kg N ha⁻¹ to patches in the pasture (Snow et al.,
2013), which far exceeds the plant nutritional requirements and can result in significant
nitrate leaching into the soil below the root zone and to ground water (Ledgard and Saunders,
1982). Leaching is accelerated when soil NO₃-N levels accumulate before periods of high
rainfall and/or when the soil texture is sandy (Cuttle et al., 1998; Fillery, 2001).
In grass-legume pasture systems, the uptake of soil nitrate by companion grasses can serve to reduce inorganic soil N levels, help maintain high levels of BNF and decrease the amount of nitrate lost from the system via leaching. Despite the fact that much research has been conducted on perennial ryegrass-white clover mixtures grazed by sheep (Ovis aries) and dairy cattle (Bos taurus) in Australia, New Zealand and Europe, less has been reported in the United States. Tall fescue (Lolium arundinaceum Shreb.; TF) is the dominant cool season perennial grass grown in the southeastern US, and little information is available regarding its compatibility to clover in regards to soil N cycling. Also, the grazing behavior and selectivity of goats (Capra hircus) is very different than that of sheep and cattle. Goats do not choose a diet as high in legumes as the other livestock and therefore may have a different effect on the sward botanical composition and N fixing capability (Clark et al., 1982). The objective of this study was to measure the effects of N source and grazing goats/excreta on biological N fixation, forage N yield and soil N cycling in tall fescue and clover pasture ecosystems.

**Materials and Methods**

*Experimental Site and Design*

Plots were planted in September 2011 at the North Carolina State University Lake Wheeler Field Laboratories in Raleigh, NC. The soils were Appling fine sandy loam and Cecil gravelly sandy loam. The trial was replicated over 3 years (2012-2014). The experimental design was a split block with three field replicates and four main N treatments: TF mixed with RC (TFRC), TF mixed with WC (TFWC), TF fescue fertilized with N
(TFPOS) and TF with no additional N (TFNEG). Each plot measured 0.2 ha. Within each main treatment plot, there were grazed (G) and mowed (M) subplots at 2 sampling locations at either end of the plot. Grazing subplots measured 155 m$^2$ while mowed subplots measured 78 m$^2$.

**Pasture Establishment**

In August 2011, the existing forage at the research site (‘Max Q’ TF and ladino WC) was sprayed with Roundup® and then incorporated. ‘Max Q’ TF was planted with a no-till drill (Truax Company, New Hope, MN) at the rate of 19.6 kg ha$^{-1}$ over the entire prepared field bed in September 2011. A mixture of ‘Cinnamon Plus’ and ‘Kenland Red’ RC was immediately overseeded at 10.7 kg ha$^{-1}$ in September 2011 and again in February 2014. Cinnamon Plus was developed for better persistence under grazing pressure. ‘Will’ ladino WC was overseeded at 4.4 kg ha$^{-1}$ in September 2011. This variety was developed at NCSU and is noted for persistence in hot climates. Because ladino WC had been growing in the field for one year previous to planting, it was assumed native rhizobia bacteria were in the soil. TFPOS plots were fertilized at 56 kg N ha$^{-1}$ as ammonium sulfate in February and September each year. The plots were cut for hay once during each summer. In March 2012 before the start of the trial, the grass-only plots were sprayed with Weedmaster® to kill any volunteer clover. Plots were fertilized with phosphorus (58 kg P ha$^{-1}$) and potassium (149 kg K ha$^{-1}$) in September 2012 based on soil test results. No additional P of K was needed for the duration for the trial as per annual soil tests.
Animals and Grazing Management

The protocol for this study was approved by the Institutional Animal Use and Care Committee of North Carolina State University. Boer-cross and Kiko-cross lactating does with their twin kids were grazed during the springs (March – May) of 2012, 2013 and 2014. Does were assigned to one of 12 groups based on doe age, kid age and kid breed composition in an effort to equalize all groups. The groups were then randomly assigned to replicate and treatment. Initial bodyweight of does were 48.5 kg ± 8.7 (2012), 51.2 kg ± 9.4 (2013) and 47.1 kg ± 8.0 (2014). The average age (d) and weight (kg) of the kids at the beginning of each trial was as follows: 2012- 17.8, 6.8; 2013 - 21.5, 8.2 and 2014 - 26.6, 6.7, respectively. Grazing began in late March and lasted for 56 d. Stocking density varied per plot per year based on available forage with additional put and take animals used to equalize stocking density to approximately 500 kg forage ha⁻¹ per doe (forage allowance of 4.3 kg forage d⁻¹).

During fall 2012 (September – November), eight month old wethers (n = 5 per plot with the exception of TFWC: n= 3.3/plot; initial BW 23.0 kg ± 6.7) were stratified by weight and then randomly assigned to treatment. They were only grazed for 39 d due to the lack of forage production. Additional put and take animals were used to equalize stocking density to approximately 450 kg forage ha⁻¹ per wether (forage allowance of 3.1 kg forage d⁻¹).

Animals were moved to a fresh strip of forage (155 m²) every two days (spring) or 2.5 days (fall) resulting in a grazing cycle length of 23 days (spring) and 29 days (fall). Animals were given a mineral supplement (Cattleman’s Pride Weathershed 2:1 Beef Mineral; Southern States Cooperative, Inc., Richmond, VA) three times a week at the rate of 28 g hd⁻¹ d⁻¹. Forage in the mowed subplots was harvested with a walk behind sickle bar
mower (Jari Mowers, Mankato, MN) at the same time animals were grazing the corresponding G subplots. Forage was mowed to an average height of 7.5 cm in an effort to match post-grazing height. Cut forage was then removed from the plot to prevent nutrient recycling.

Forage Sampling and Analysis

Estimates of forage mass for treatment effects were determined in the G and M subplots (4 per plot) for each grazing cycle using a 0.25m$^2$ falling plate meter three days before grazing/mowing. Ten height measurements were taken in the G subplots and 5 in the M subplots. Six sample sites (0.25m$^2$) were clipped to 5.0 cm for each treatment and each harvest type representing the range of forage mass present. Two sites represented low forage mass, two sites represented medium forage mass and two sites represented high forage mass. Clipped forage was dried at 60° C for 48 hours and weighed hot to determine DM yield (kg ha$^{-1}$). The equation developed from the linear regression of plate meter height against DM yield was used to predict available forage mass before grazing. These equations were used to estimate forage mass for determination of stocking density every 4 d. Calibrations were repeated for each grazing cycle. Post-harvest forage mass was determined using the same procedure.

Forage was sampled for determination of nutritive value in the G and M subplots before each grazing/mowing event. Forage was clipped 5 cm from ground level at 12 randomly selected sites within G subplots and 6 sites from M subplots and then hand separated into four fractions consisting of green TF, brown/dead TF, clover and ‘other’
species. Fractions were then dried at 60° C for 48 hours and weighed hot to determine the percent of each fraction in the sward on a DM basis.

Fractions were ground through a 1-mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and stored in whirl-pak bags (NASCO, Modesto, CA) at room temperature until analyzed. Dry matter, Kjeldahl nitrogen, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by AOAC procedures (1999). The NDF, ADF and lignin were determined sequentially according to Van Soest et al. (1991) using the ANKOM200 Fiber Analyzer (Ankom Corp., Fairpark, NY). Cellulose was calculated as the difference between ADF and the 72% sulfuric acid residue. Total N was determined by auto analyzer (Technicon Industrial Systems, Tarrytown, NY).

**Biological Nitrogen Fixation of Clovers**

The %N derived from the atmosphere (Ndfa) of white and red clover was determined using the $^{15}$N Natural Abundance technique and the following equation (Shearer and Kohl, 1986):

$$ \%\text{Ndfa} = \frac{\partial^{15}\text{N reference (fescue)} - \partial^{15}\text{N fix (legume)}}{\partial^{15}\text{N reference} - B} $$

The proportion of $^{15}$N from a non-fixing reference plant (TF) grown in the same treatment plot as the legume (clover) is represented by $\partial^{15}$N reference. The reference plant should have access to the same soil N pool as the legume. The proportion of $^{15}$N in the legume is represented by $\partial^{15}$N fix. The B value is the isotopic fractionation value of the legume shoot tissue that is 100% dependent on BNF. B-values are usually in the range of -1
to -2% (Shearer and Kohl, 1986). White clover B values of -1.2 to -1.4% have been used in other published studies (Hogh-Jensen and Schjoerring, 1997; Eriksen and Hogh-Jensen, 1998). For the current study, a B-value of -1% was used based on legume work done by researchers at North Carolina State University research stations (Parr et al., 2011). Subsamples of 3.0 to 4.0 mg of dried and ground clover shoot tissue and 5.0 to 6.0 mg of dried and ground fescue shoot tissue from the grazed and mowed subplots were weighed into ultra lightweight tin capsules (Elemental Microanalysis Limited, Cambridge, UK) and sent to Stable Isotope Facility (UC Davis, Davis, CA) for $\delta^{15}$N analysis.

**Inorganic soil nitrogen**

Soil samples were taken in the grazed and mowed subplots before harvest, immediately after harvest, and approximately 7 and 14 d later for each spring grazing cycle. Soil was sampled twice over the summer months. In fall 2012, soil was sampled similarly to the spring during the 39-d grazing cycle. In fall 2013 and 2014, soil was only sampled once per subplot in October/November when forage samples were taken. Fourteen core samples per grazed subplot and seven cores per mowed subplot were taken with a 2 cm diameter soil probe to a depth of 10 cm. Samples were stored at 4°C for 24 to 48 hours before they were mixed and extracted wet using 1 M KCl and shaken for one hour. Samples were filtered through #42 Watman filter papers. Extracts were frozen until analyzed for NH$_4$-N and NO$_3$-N on a QuikChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland, CO).
Statistical Analysis

Analysis of variance using the PROC GLM procedure of SAS, version 9.3 (SAS Institute, 2008) was used to test the effects of N treatment, harvest type, year, N treatment x harvest type, N treatment x year and harvest type x year for BNF and forage characteristics. The LSMEANS option was used to generate individual treatment means. Treatment means were further separated using the Fisher’s least significant difference test (Steel et al., 1997). Soil N values were transformed (NH$_4$ - cubic root; NO$_3$ – square root) to normalize distribution before data were analyzed using the PROC GLIMMIX procedure. Data were back transformed to get the least square means that are reported. Significance was declared at $P < 0.05$.

Results and Discussion

%N derived from atmosphere (Ndfa)

During the 2012 and 2013 spring season, there was no difference in % Ndfa between the RC and WC (avg = 82.1%; range = 71 to 93%). The $\delta^{15}$N values for the legumes ranged from -0.94 to 0.02. The $\delta^{15}$N values for the TF reference plant ranged from 0.27 to 5.23. The $\delta^{15}$N of N fixing legumes are generally lower than the non-fixing reference plants, because they are utilizing less soil N. The % Ndfa of RC and WC monocultures have been estimated at 90 and 84%, respectively (Hogh-Jensen and Schoerring, 2001), whereas WC reportedly fixed 80% of its total N in ryegrass mixtures (Hogh-Jensen and Schoerring, 1997).

The % Ndfa for WC was less in fall than spring (46.3% vs 82.1%; SEM 4.19; Figure 3.1; $P < 0.05$). The $\delta^{15}$N values for the WC ranged from -0.95 to 2.01. The $\delta^{15}$N values for
the TF reference plant ranged from 0.44 to 2.98. There was no RC growth to sample. A review by Ledgard (2001) found % Ndfa of grazed WC ranged from 44 to 82% while mowed WC was greater at 80 to 98% Ndfa. The current study did not find any difference between grazed and mowed treatments. The fibrous morphology of TF roots may explain the preferential N uptake, thus maintaining low soil N and similar %Ndfa between grazed and mowed treatments.

A correlation between %Ndfa and soil nitrate-N was observed in the grazed subplots of WC in 2012 (r = - 0.71; Figure 3.2a; P < 0.01) and 2013 (r = -0.60; Figure 3.2b; P < 0.05). As the soil NO$_3$-N values increased from spring to fall, the %Ndfa decreased. Urine has been reported to decrease the % Ndfa of clovers by 37 to 70% over a 4 to 6 week period (Eriksen and Hogh-Jensen, 1998; Menneer et al., 2003; Vinther 1998). Menneer et al. (2003) found soil NH$_4$-N increased to 355 kg N ha$^{-1}$ within 3 days of homogeneously applying urine to ryegrass-WC mixtures. Subsequently, NO$_3$-N values reached levels of 314 kg N ha$^{-1}$ within 17 d and stayed high for 161 d. The current study’s authors hypothesized that there would be an immediate decrease in %Ndfa after each 23-d grazing cycle due to the N excreted in the feces and urine and subsequent increase in soil N values. That was proven incorrect. The TF planted with the clovers was growing so rapidly during the spring months, it kept the inorganic soil N levels low forcing legumes to continue fixing N at a high rate. An increase in soil NO$_3$-N did not happen until the summer months when there was less forage growth as will be discussed in detail in one of the next sections.
**Biologically fixed N**

A difference in the production of biologically fixed N (kg fixed N ha\(^{-1}\)) was observed between 2012 and 2013 and between spring and fall \((P < 0.05)\). In spring 2012, there was no difference in biologically fixed N (kg ha\(^{-1}\)) between WC and RC species (111 vs 62 kg N ha\(^{-1}\), respectively, SEM 13.0). The mean proportion of WC and RC in the fescue pastures were 40 and 30%, respectively. According to Houg-Jensen and Schjoerring (1997) WC (at 20% sward DM) in a perennial ryegrass mixture fixed 83 kg N ha\(^{-1}\). Hatch et al. (2007) estimated that a pasture containing 70% RC fixed 200 kg N ha\(^{-1}\). In spring 2013, TFWC produced a greater amount of fixed N than TFRC (75 vs 15 kg N ha\(^{-1}\), respectively; SEM = 3.1; \(P < 0.05\)).

The amount of N fixed by a legume is largely dependent upon legume DM production and %Ndfa. In the current study, forage DM yield declined from 2012 to 2013 (data not shown; \(P < 0.05\)). During the spring, the proportion of white clover in the sward was similar in 2012 and 2013 at 40.4% and 43.2%, respectively but declined to 18.7% in 2014. The proportion of red clover declined from 30.0% in 2012 to 11.5% in 2013 and 2.6% in 2014. The amount of biologically fixed N followed this pattern. For every metric tonne of clover DM, RC fixed 30 to 31 kg N and WC fixed 31 to 40 kg N. This is in agreement with a study conducted by Ledgard et al. (2001b) in which WC mixed with perennial ryegrass and grazed by dairy cattle fixed 36 kg N metric tonne\(^{-1}\) of clover DM. Others have estimated 49 to 63 kg fixed N metric tonne\(^{-1}\) clover DM (Elgersma and Hassink, 1997). It has also been noted that the amount of N fixed in plant tissue below the grazing height (stolons and roots) can account for an additional 70% of the total N fixed (Jorgensen and Ledgard, 1997).
During fall 2012 and 2013 there was no RC to sample, and WC only fixed 10 and 1 kg N ha\(^{-1}\), respectively. These low values are the result of the 43% decrease in % Ndfa and 75% decline in WC DM proportion in the sward from spring to fall. Vinther (1998) calculated white clover fixed 50% of its N within the first 2 to 3 months of the growing season. Ledgard et al. (2001) reported that white clover fixed 61 to 72% of its N during the spring and summer months.

The only difference in the total production of biologically fixed N (kg fixed N ha\(^{-1}\)) between harvest types occurred in spring 2012 as the clovers in mowed treatments fixed a greater amount of N than those in grazed treatments (80 vs 69 kg N ha\(^{-1}\); SEM = 1.56; \(P < 0.05\)). This is logical, because the proportion of clover DM in spring 2012 was almost 10% greater in mowed plots than in grazed plots. Also, in mowed systems, less forage N is recycled therefore depleting soil N and forcing the clover to continue fixing N at a high rate. A review of 20 years of field studies by Ledgard (2001) estimated an average of 66 to 152 kg of fixed N ha\(^{-1}\) from mowing trials, whereas long term grazed, permanent pastures yielded only 48 to 59 kg N ha\(^{-1}\) yr\(^{-1}\) due to lower legume content. Moderately-severe defoliation of mixed ryegrass-white clover swards led to a 36% increase in annual N\(_2\) fixation compared to light defoliation (Menneer et al., 2003).

Forage Nitrogen Yield

The total amount of forage N (kg ha\(^{-1}\)) produced per treatment is presented in Figure 3.3 a,b as a treatment by year interaction (\(P < 0.01\)) was detected. The TFWC produced the greatest amount of forage N in spring 2012 and 2013 averaging 217 and 204 kg N ha\(^{-1}\),
respectively ($P < 0.001$). Due to the decrease in the proportion of clover in the stand in 2014, forage N yield for TFWC was similar to TFPOS for that year. Forage N yield for TFRC was lower than for TFWC each year and lower than POS in 2013 and 2014 ($P < 0.001$). During the fall of 2012 and 2013, the TFPOS treatment produced the greatest amount of forage N averaging 115 and 58 kg N ha$^{-1}$, respectively ($P < 0.05$). Elgersma et al. (2000) reported annual N yields from perennial ryegrass-WC swards (ranging from 231 to 516 kg N ha$^{-1}$) were greater than perennial ryegrass fertilized with 150-180 kg N ha$^{-1}$ (168 to 197 kg N ha$^{-1}$).

In 2012, there was a tendency ($P < 0.10$) for the grazed plots to produce more forage N than the mowed plots in both spring and fall. During the spring 2013, grazed plots produced a greater amount of forage N than mowed plots ($P < 0.05$).

**Forage nitrogen transfer from animal to plant**

Nitrogen can cycle through a pasture either above ground via excreta of grazing animals or below ground through decomposition and mineralization of plant material as well as through rhizodeposition. Both pathways result in increased soil N pools for forage uptake.

In order to quantify the amount of N that was transferred above ground via excreta to TF in the current experiment, the difference method and the following equation was used:

$$\text{Transferred forage N} = \frac{(\text{total N yield in the grazed plots} - \text{total N yield in mowed plots})}{\text{total N yield in the grazed plots}}$$
Calculations from data in Table 3.1 indicate the TF-only treatments depended more on excreta for N cycling in the pastures than the grass-legume plots. The 3 year mean of the percentage of N transferred via excreta during spring for TFRC, TFWC, TFPOS and TFNEG was 9.7, 4.9, 20.2 and 21.8%, respectively. The grass N yield of the grass-clover treatments was arithmetically greater than the grass N yield of the negative control suggesting greater below ground N cycling. While it is difficult to know exactly what caused this forage N increase in mixed treatments (plant decomposition, mineralization, rhizodeposition), greater inorganic soil N levels in the grass-legume pasture were also measured (which is discussed in detail in a later section).

**Forage characteristics**

Cellulose and Lignin

Measurements of the cellulose and lignin concentration of each forage species and each treatment sward were made during spring season only. A year by forage species interaction was observed ($P < 0.01$) for concentrations of cellulose and lignin, with TF plants having greater cellulose but less lignin than either RC or WC in all 3 years ($P < 0.01$; Table 3.2). The RC and WC plants had similar cellulose concentrations for all three years and similar lignin concentrations during 2012 and 2013.

Treatment plots containing WC had the lowest cellulose concentrations, and the grass only swards had the greatest cellulose concentrations ($P < 0.01$; Figure 3.4). The cellulose concentration of TFRC treatments was closely linked to the percentage of RC in the sward and was similar to TFPOS in 2013 and 2014. Even though the clover plants had greater
lignin than TF, this did not affect the lignin concentrations in swards ($P = 0.29$). The three year means of TFWC, TFRC, TFPOS and TFNEG were 3.26, 3.22, 3.17 and 2.75% lignin, respectively).

Lignin:N

The lignin:N ratio of the RC and WC species were similar to each other and lower than that of TF in 2012 and 2013 ($P < 0.01$; Table 3.2). The sward lignin:N ratios were similar between treatments in 2012 and 2014 and ranged from 0.89 and 1.23. The TFWC had a lower ratio in 2013 than TFNEG, TFPOS and TFRC (1.02, 1.41, 1.44 and 1.60, respectively; $P < 0.01$). Thomas and Asakawa (1993) reported a linear correlation between lignin:N ratio and the decomposition loss of N and organic matter from grass and legume litter.

C:N

There was a forage by year interaction ($P < 0.001$) for the C:N ratio of the individual forage species as well as a treatment by year interaction ($P < 0.001$) for the sward C:N ratio. The RC and WC species had similar C:N ratios, and were lower than TF for all 3 years (Table 3.2) These values did affect the C:N ratio of the swards as TFWC and TFRC were less than TFPOS and TFNEG in 2012 (14.7, 16.4, 20.2 and 23.7, respectively; $P < 0.001$; Figure 3.5). The TFWC continued to have a lower ratio than all other treatments through 2013 and 2014. Ranells and Wagger (1996) reported that lower C:N ratios of rye – hairy vetch (*Secale cereal* - *Vicia villosa*) cover crop mixtures compared to rye monocultures (14-21:1; 40:1, respectively) resulted in a greater amount of N released during 8 wk of decomposition. Likewise, a linear correlation ($r > 0.91$) between net N mineralization and
C:N ratio has also been documented for perennial ryegrass and WC (deNeergaard et al., 2002).

**Inorganic Soil Nitrogen**

Inorganic soil N (NO$_3$-N and NH$_4$-N) values are presented by year in Table 3.3. Mean ammonium-N values increased each year from 2012 to 2014 ($P < 0.01$). Grazed values were consistently greater than mowed values ($P < 0.01$). Immediately after each grazing event, the level of soil NH$_4$ spiked and then declined within 7 d (Figure 3.6). In the 2012 grazed subplots, TFWC had similar NH$_4$-N value compared to TFRC, and TFWC was greater than the TF-only treatments. The TFWC and TFRC were also greater than TFPOS and TFNeg in the 2012 mowed subplots ($P < 0.05$). In 2013, TFWC had greater NH$_4$-N than all other treatments ($P < 0.05$) in G and M subplots, but TFRC was similar to the fescue only treatments due to the decrease in RC proportion. By 2014, there were no treatment differences in either the G or M subplots. There are few published reports of inorganic soil N measurements under grazed pastures, but Vines et al. (2006) documented greater mean soil NH$_4$-N levels under TF pastures containing 31% RC compared to TF pastures fertilized with 160 kg N ha$^{-1}$. In that study, the average NH$_4$-N level of grazed TF-legume and fertilized TF pastures was 11.9 mg/kg which is slightly higher than the range of 2.4 to 6.8 mg kg$^{-1}$ measured in the current study.

Unlike soil NH$_4$ values, mean soil NO$_3$-N values were similar between years. Data are presented separately for each year due to differences in grazing management and varying clover percentages in the sward (Table 3.3). For each year, soil nitrate values stayed low
during the spring season, but rose through summer and fall (Figure 3.7; $P < 0.05$). Mineral soil N is notably lower during the spring season due to the high demand by rapidly growing forages for N and greater in the fall and early winter as forage growth declines (Peoples and Baldock, 2001). The nitrate levels in grazed treatments were greater than those in mowed treatments ($P < 0.01$). Within the grazed subplots, TFWC and TFRC had similar nitrate levels in 2012 and greater than those of TF-only treatments ($P < 0.5$) probably due to the high proportion of clover in both treatments. The TFWC was also greater than the TF-only treatments in 2013 and 2014 ($P < 0.05$). The nitrate levels of TFRC and TFWC were greater than for the TFPOS and TFNEG in mowed treatments in 2012 and 2013, but not in 2014 as the clover proportion declined. Vines et al. (2006) documented that soil nitrate levels of Tc-RC pastures were lower than fertilized TF pastures and never exceeded 3 mg kg$^{-1}$. In the current study, soil nitrate levels never exceeded 4 mg kg$^{-1}$.

The increased levels of inorganic soil N in the mowed legume plots compared to mowed grass plots can be attributed partly to rhizodeposition which includes root exudates, sloughed off root tissue and dead roots. Estimates of RC, WC and ryegrass monocultures, have been documented at 640, 710 and 90 kg rhizodeposited N ha$^{-1}$, respectively, while mixtures of ryegrass and clover produced 320 to 890 kg N ha$^{-1}$ (Hogh-Jensen and Schjoerring, 2001). Ledgard (1991) found that the below ground N transfer was largest during dry summer conditions possibly due to sloughing of nodules. In the current study, soil NO$^3$-N levels began to rise during the summer months.
Conclusion

The current research studied the impacts of grazing and clovers on N cycling in TF pastures. Soil N values were affected by grazing as NH$_4$ levels increased immediately after each grazing event. Contrary to other published work, this did not lead to a quick increase in soil nitrate levels because the fast growing companion grass took up excess soil N. Other studies have applied urine at a high N rate homogeneously across the entire test plot area in contrast to the spatial application by the goats in the current study. Our intention, through intensive sampling, was to capture the variability of %Ndfa of clovers based on the heterogeneous excreta deposits by the goats. Soil nitrate levels rose from spring to fall and caused a severe decline in biological nitrogen fixation. Urine and feces excretions played a greater role in N transfer from animal to plant in the grass-only plots as compared to the mixed pastures. The clover component of the mixed plots contributed to a lower sward C:N ratio indicative of greater N mineralization and nutrient cycling. Overall, adding RC or WC to TF pastures in rotational grazing systems for goats increased the rate and quantity of forage N cycling in pasture ecosystems and can reduce or eliminate the need for N fertilization.

Implications

This study showed a very distinct annual pattern of inorganic soil N values in a pasture system and the patterns impact on BNF. When soil NH$_4$-N and NO$_3$-N are low in the spring, BNF is high. As NO$_3$-N increases through the summer and fall, BNF decreases significantly. The amount of N fixed by legumes is dependent upon %Ndfa and forage mass.
Under optimal conditions, WC can fix over 100 kg N ha\(^{-1}\), which is sufficient for TF pasture growth. The majority will be added to the system during the first half of the year. The TF-clover pastures had greater soil N values due to the recycling of the high N excreta and the decomposition of high N forages. Unfortunately, if this inorganic soil N is not captured by plant material, it can be lost through nitrate leaching. Harvesting hay from these mixed pastures through the summer and fall instead of grazing them may help to decrease the soil N levels.
REFERENCES


Table 3.1. Nitrogen yield (kg N ha\(^{-1}\)) of tall fescue pastures with varying nitrogen sources that were either grazed by goats (G) or mowed (M) during spring 2012, 2013 and 2014.

<table>
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<th>TFRC -G</th>
<th>TFRC -M</th>
<th>TFWC -G</th>
<th>TFWC -M</th>
<th>TFPOS -G</th>
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<td>86</td>
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<td>20</td>
<td>60</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N Yield</td>
<td>75</td>
<td>76</td>
<td>127</td>
<td>134</td>
<td>137</td>
<td>117</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Grass N Yield</td>
<td>72</td>
<td>71</td>
<td>92</td>
<td>77</td>
<td>137</td>
<td>117</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Clover N Yield</td>
<td>3</td>
<td>5</td>
<td>35</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized fescue); TFNEG = negative control (fescue no N)
Table 3.2 Chemical composition (% cellulose DM, % lignin DM, lignin:N, C:N) of red
clover, white clover and tall fescue species within three spring (March – May) grazing
seasons (treatment x year interaction $P < 0.01$)

<table>
<thead>
<tr>
<th>Year</th>
<th>Forage</th>
<th>Cellulose</th>
<th>Lignin</th>
<th>Lignin:N</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>Red Clover</td>
<td>13.65$^b$</td>
<td>3.50$^a$</td>
<td>0.83$^b$</td>
<td>10.92$^b$</td>
</tr>
<tr>
<td></td>
<td>White Clover</td>
<td>13.72$^b$</td>
<td>3.52$^a$</td>
<td>0.93$^b$</td>
<td>11.79$^b$</td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>27.38$^a$</td>
<td>2.33$^b$</td>
<td>1.10$^a$</td>
<td>21.16$^a$</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.33</td>
<td>0.68</td>
<td>0.033</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>$P$-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2013</td>
<td>Red Clover</td>
<td>14.42$^b$</td>
<td>4.42$^a$</td>
<td>0.85$^b$</td>
<td>8.84$^b$</td>
</tr>
<tr>
<td></td>
<td>White Clover</td>
<td>15.33$^b$</td>
<td>5.09$^a$</td>
<td>1.35$^b$</td>
<td>12.82$^b$</td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>31.00$^a$</td>
<td>3.38$^b$</td>
<td>1.41$^a$</td>
<td>18.83$^a$</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.72</td>
<td>0.26</td>
<td>0.081</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>$P$-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2014</td>
<td>Red Clover</td>
<td>16.69$^b$</td>
<td>8.25$^a$</td>
<td>1.90$^a$</td>
<td>9.44$^b$</td>
</tr>
<tr>
<td></td>
<td>White Clover</td>
<td>14.75$^b$</td>
<td>4.80$^b$</td>
<td>1.05$^b$</td>
<td>10.48$^b$</td>
</tr>
<tr>
<td></td>
<td>Tall Fescue</td>
<td>28.27$^a$</td>
<td>3.18$^c$</td>
<td>1.17$^b$</td>
<td>16.62$^a$</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.57</td>
<td>0.52</td>
<td>0.14</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>$P$-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a-b means with differing superscript differ within columns
Table 3.3 Mean annual inorganic soil NO\textsubscript{3}-N and NH\textsubscript{4}-N values (mg kg\textsuperscript{-1}) of tall fescue pastures with varying N sources that were either grazed by goats or mowed.

<table>
<thead>
<tr>
<th>Year</th>
<th>Forage†</th>
<th>NO\textsubscript{3} -N</th>
<th>NO\textsubscript{4} -N</th>
<th>NH\textsubscript{4} -N</th>
<th>NH\textsubscript{4} -N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Graze</td>
<td>Mow</td>
<td>Graze</td>
<td>Mow</td>
</tr>
<tr>
<td>2012</td>
<td>TFRC</td>
<td>1.98\textsuperscript{ab}</td>
<td>1.75\textsuperscript{ab}</td>
<td>3.75\textsuperscript{ab}</td>
<td>1.92\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>TFWC</td>
<td>3.04\textsuperscript{a}</td>
<td>2.45\textsuperscript{a}</td>
<td>5.16\textsuperscript{a}</td>
<td>3.26\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>1.46\textsuperscript{b}</td>
<td>0.82\textsuperscript{b}</td>
<td>2.43\textsuperscript{b}</td>
<td>0.85\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>1.25\textsuperscript{b}</td>
<td>0.85\textsuperscript{b}</td>
<td>2.73\textsuperscript{b}</td>
<td>1.43\textsuperscript{c}</td>
</tr>
<tr>
<td>2013</td>
<td>TFRC</td>
<td>1.08\textsuperscript{b}</td>
<td>1.20\textsuperscript{ab}</td>
<td>2.82\textsuperscript{b}</td>
<td>2.08\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>TFWC</td>
<td>3.94\textsuperscript{a}</td>
<td>2.23\textsuperscript{a}</td>
<td>6.85\textsuperscript{a}</td>
<td>6.14\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>1.28\textsuperscript{b}</td>
<td>0.84\textsuperscript{b}</td>
<td>3.39\textsuperscript{b}</td>
<td>1.52\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>0.92\textsuperscript{b}</td>
<td>0.82\textsuperscript{b}</td>
<td>2.76\textsuperscript{b}</td>
<td>2.09\textsuperscript{b}</td>
</tr>
<tr>
<td>2014</td>
<td>TFRC</td>
<td>1.38\textsuperscript{b}</td>
<td>1.22\textsuperscript{a}</td>
<td>4.44\textsuperscript{a}</td>
<td>2.87\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>TFWC</td>
<td>2.81\textsuperscript{a}</td>
<td>1.74\textsuperscript{a}</td>
<td>5.27\textsuperscript{a}</td>
<td>3.94\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>TFPOS</td>
<td>1.66\textsuperscript{b}</td>
<td>0.86\textsuperscript{a}</td>
<td>6.32\textsuperscript{a}</td>
<td>2.78\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>TFNEG</td>
<td>1.00\textsuperscript{b}</td>
<td>0.89\textsuperscript{a}</td>
<td>5.02\textsuperscript{a}</td>
<td>3.40\textsuperscript{a}</td>
</tr>
</tbody>
</table>

† TFRC = tall fescue-red clover; TFWC = tall fescue-white clover; TFPOS = positive control (N fertilized fescue); TFNEG = negative control (fescue no N)

a-c means with differing superscript differ within columns
**Figure 3.1** Percentage of nitrogen derived from the atmosphere (% Ndfa) by white clover during three spring grazing cycles and two fall grazing cycles during 2012 and 2013.

* $P < 0.05$ spring vs fall
**Figure 3.2** Correlation between soil NO$_3$-N levels and % nitrogen derived from atmosphere (%Ndfa) of white clover in tall fescue-white clover pastures during 2012(a) and 2013(b)
Figure 3.3 Seasonal forage nitrogen yield (kg N ha\(^{-1}\)) of tall fescue pastures with either white clover (WC), red clover (RC), nitrogen fertilization (POS) or no additional N (NEG) during spring 2012, 2013 and 2014 (a) or fall 2012 and 2013 (b) grazing seasons

* P <0.05 ** P < 0.00
Figure 3.4 Cellulose concentration (% DM) of for tall fescue pastures with either white clover (WC), red clover (RC), nitrogen fertilization (POS) or no additional N (NEG) during spring 2012, 2013 and 2014 grazing seasons.

*Treatment effect $P < 0.01$
Figure 3.5. Carbon:nitrogen (C:N) ratio for tall fescue pastures with either white clover (WC), red clover (RC), nitrogen fertilization (POS) or no additional N (NEG) during spring 2012, 2013 and 2014 grazing seasons. *** Treatment effect $P < 0.001$
Figure 3.6 Soil NH₄-N levels for tall fescue pastures with either white clover (WC), red clover (RC), nitrogen fertilization (POS) or no additional N (NEG) during 2012 and either grazed by goats (a) or mowed (b). Arrow indicates measurement was taken 1 day after grazing/event.
Figure 3.7 Soil NO$_3$-N levels (avg 2012-2014) for tall fescue pastures with either white clover (WC), red clover (RC), nitrogen fertilization (POS) or no additional N (NEG) and either grazed by goats (a) or mowed (b).
APPENDICES
Appendix A. Plot design

Sample Graze Plot → Sample Mowed Plot → Sample Mowed Plot → Sample Graze Plot

Plot 12 – POS
Plot 11 – NEG
Plot 10 – WC
Plot 9 – RC
Plot 8 – WC
Plot 7 – NEG
Plot 6 – RC
Plot 5 – POS

Plot 4 – Max Q Fescue (NEG)
Plot 3 – Max Q Fescue & ‘Will’ White Clover (WC)
Plot 2 – Max Q Fescue & 112 kg N/ha Fertilizer (POS)
Plot 1 – Max Q Fescue & ‘Kenland/Cinnamon Plus’ Red Clover (RC)

Plot = 0.2 ha  Animals moved to new forage every 2 days