8-2012

The Impact of Selected Forage Legumes on Cattle Performance, Forage Production, and Soil Quality, and Evaluation of Legume Persistence under Grazing

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Citation

Briggs, B. E. (2012). The Impact of Selected Forage Legumes on Cattle Performance, Forage Production, and Soil Quality, and Evaluation of Legume Persistence under Grazing. *Graduate Theses and Dissertations* Retrieved from https://scholarworks.uark.edu/etd/529

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The Impact of Selected Forage Legumes on Cattle Performance, Forage Production, and Soil Quality, and Evaluation of Legume Persistence under Grazing

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

By

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University of Arkansas
Bachelor of Science in Animal Science, 2009

August 2012
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Abstract

Interest in substituting legumes for N fertilizer in beef cattle grazing systems has recently increased with rising fertilizer prices. Legumes are well known for their ability to fix atmospheric N and decrease dependence on input of N fertilizer. However, there are still difficulties associated with legume utilization including establishment and persistence. Two experiments were conducted to evaluate legume performance under herbivory. The objective of Experiment 1 was to compare forage production and beef cattle gains from annual ryegrass [*Lolium multiflorum* (L.)] and bermudagrass [*Cynodon dactylon* (L.) Pers.] pastures fertilized with N or overseeded with legumes. Gelbvieh × Angus crossbred heifers (n = 40; average of 264 ± 45.62 kg initial BW) were assigned to one of eight, 2-ha pastures in the spring of each of the three years of the study. All pastures were overseeded with ‘Marshall’ annual ryegrass, and were not seeded with any clover (Con) or overseeded with ‘Dixie’ crimson clover [(C; *Trifolium incarnatum* (L.)], ‘Osceola’ white clover [L; *Trifolium repens* (L.)], or a combination of crimson clover and white clover (CL). Grazing initiated early- to mid-spring and continued until early- to mid-May. Total body weight (BW) gain was greater ($P < 0.05$) in the spring season for Con compared to the legume treatments. However, average daily gain (ADG) was not different ($P > 0.05$) in spring, and there were no differences ($P > 0.05$) in total BW gain or ADG in summer. Although clovers may not be able to entirely eliminate the need for N fertilizer, they may help reduce dependency on it by aiding in the production of cattle having similar BW gains to cattle grazing traditionally fertilized pastures. The objective of Experiment 2 was to monitor the persistence of three annual and three perennial legume species overseeded into common bermudagrass pastures that were rotationally stocked. The three annual species were crimson clover (cv. Dixie), arrowleaf clover [*Trifolium vesiculosum* (Savi), cv. Yucchi], and hairy vetch [*Vicia villosa* (Roth), cv. VNS]. The three perennial species were white clover (cv. Durana), red clover [*Trifolium pretense* (L.), cv. Cinnamon Plus], and alfalfa [*Medicago sativa* (L.), cv. Ameristand 403T]. Annual clovers were managed to reseed themselves. Crimson clover persisted two years and all other annul species for three years. Among perennial legumes, only white and red clovers persisted for three years, while alfalfa stands disappeared after the second year of the study. The frequency of occurrence of weeds and other undesirable plants generally increased each year while legume populations declined in all six clover treatments. In order to maintain healthy and dense legume populations in grazing systems, it may be necessary to develop and adopt aggressive weed control strategies using chemical compounds including improved grazing management strategies.

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Acknowledgements

I would like to thank Dr. Dirk Philipp for his wise counsel in all manner of things throughout the course of my graduate studies including academics.

I would also like to thank Dr. Coffey and Dr. Savin for serving on my committee and augmenting my studies with their own expertise.

I am extremely grateful for Don Hubbell, John Tucker, Rick Wilson, Todd Coles, Henry Headley, Tom Hess, and Joe Rolins in Batesville and Greg “G-money” Montgomery and all his staff in Monticello. They made this research possible and kept my trips to Batesville and Monticello fun and interesting.

I also greatly appreciate the help of Karen Anschutz, Doug Galloway, and the other laboratory staff. Without their help I would probably still be doing laboratory work instead of writing this thesis.

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Introduction

During the past centuries, soil nutrient removal occurred on such a small scale that nutrients were returned to the soils in sufficient levels by humans and animals via waste products and other forms of decaying organic matter. As civilizations began to grow in more recent centuries and populations began to expand rapidly, a much greater strain was put on the environment. Nutrients in the form of crops and grazing animals were being shipped away from farmlands to urban areas for consumption, but some nutrients were still returned in the form of human waste which was used as fertilizer—a practice that was continued in certain areas until the early 20th century. However, even with some return of nutrients to agricultural areas, nutrient deficiencies soon became a problem for emerging modern agricultural practices (Kjaergaard, 2003).

One of the most important but growth-limiting nutrients is nitrogen, which is a key component of chlorophyll and a major part of proteins (Smil, 1990). Although about 80 percent of the earth's atmosphere is comprised of N_2, plants cannot use N in the gaseous form. Plants take up N from the soil as nitrate (NO_3^-) and ammonium (NH_4^+) (Ball et al., 2007), and plants use the amine form for synthesis of necessary compounds such as nucleic acids and amino acids (Lindemann, 2003).

Today, producers use N fertilizer to provide crops and pastures with the necessary levels of N to offset crop N removal rates. According to the USDA Economic Research Service (accessed March 24, 2011), 790,357,478 Mg of urea, anhydrous ammonia, ammonia, ammonium nitrate, ammonium sulfate, N solutions, sodium nitrate, and other N containing compounds were used as fertilizer between 1960 and 2008. Synthetic N fertilizer was the cheapest way of providing crops with N until the increase of natural gas prices during the past years, a period of relative unrest in the Middle East, and China increasing tariffs on exported fertilizers (Silva, 2011). Pricing data on fertilizers provided by the USDA showed an approximate increase of 21.9% on urea, one of the more commonly used N fertilizers, between 2007 and 2008 which made applying N fertilizer a financial problem for many operations. With rising fertilizer prices, legumes have been reconsidered as a viable solution to supply cattle grazing pasture systems with N. With this thesis, the author investigated in two separate experiments the effects of selected annual and perennial legume species on cattle performance, legume persistence under grazing, soil quality, and plant-chemical composition.

Literature Review

Historical Legume Utilization

The beneficial effects of legumes have been known in at least some basic form for millennia (Bergersen, 1982). The Lake People of Switzerland who lived from 5,000 to 4,000 B. C. grew legumes including peas and a dwarf field bean (*Fabaceae*) (Whyte et al., 1953). Around 4,000 B. C., during the time of the ancient Egyptian dynasties, legumes such as lentils and faba beans [*Vicia faba* (L.)] were a part of the everyday diet (Bergersen, 1982). Chinese sources reported the cultivation of soybean between 3,000 and 2,000 B. C., and Roman writers even described the importance of legumes in terms of food and soil improvement (Whyte et al., 1953). These authors indicated further that American Indians were well known to grow beans in addition to maize crops.

Nitrogen fertilizer

Fertilizer is applied with the purpose of increasing crop production and to achieve a profit despite the additional cost. Synthetic N fertilizer has been widely adopted as part of modern farming practices to increase food and animal production and therefore increase the number of people that can be fed per unit land area. This is accomplished in two general ways. First, when fertilizer is applied at correct amounts and appropriate times, crop demand for N can be satisfied throughout the growing season. Second, N fertilizer reduces the need to find agronomic alternatives such as planting crops that increase soil fertility (Crews and Peoples, 2003; Smil, 2001). In the 1950's, up to 50% of N used for agriculture was provided by biological N fixation by legumes or green manure crops, but by the mid-1990's this percentage had decreased to approximately 20% (Smil, 2001). More recently, legumes have been considered to improve the relationship between energy costs, forage production, and animal performance in pasture grazing settings across the globe in order to maximize returns and protect natural resources (Greenquist et al., 2009).

Sources of N fertilizer

The majority of commercial N fertilizer is produced via the Haber-Bosch process (Ball et al., 2007). In addition to the 120 Tg associated with food consumption and production, approximately 25 Tg per year of N is created by the combustion of fossil fuels, and approximately 20 Tg per year is also created from the Haber-Bosch process for other uses. These numbers amount to about 165 Tg per year of reactive N produced which is twice the amount that is biologically fixed per year in natural terrestrial ecosystems (Galloway et al., 2002). An additional 100 Tg N per year that is associated with food production but is not consumed by humans is released into the environment.

Of the approximately 6.8 billion people on the planet, almost 40% are dependent on synthetically produced N fertilizer. By year 2050, up to 5.5 billion people will be dependent upon synthetic N fertilizers (Crews and Peoples, 2003). Recent measurements of total human N ingestion were approximately 20 Tg per year from food (Galloway et al., 2002).

Environmental advantages of N fertilizer

Nitrogen fertilization has had a dramatic effect on crop and forage production. Between 1965 and 1994, land area for grain production decreased from 0.2 to 0.12 ha per person in the U. S. while yields increased by 16% (Mosier et al., 2002). Approximately 40% of the large annual increase in crop production between 1960 and 1995 can be attributed to synthetic N fertilizer (Brown, 1999). Nitrogen fertilizer can be applied readily when necessary unlike legume-based N, which depends on vegetative growth throughout the growing season. Additionally, inorganic N fertilizer, because it is readily available, affects soil fertility status much more quickly than green manure or legumes which take time to decompose and transfer nitrogen back to the soil N pool.

Environmental disadvantages of N fertilizer

Nitrogen fertilizer may greatly increase crop yield, but applications can be inefficient. Average plant N recovery rates range from 40 to 50% (NRC, 1993), but some of the N taken up by plants remains in roots and other crop residues and is not included in harvested N. The amount of N actually removed during harvesting can be as low as 35% (NRC, 1993). The inefficiency of synthetic N uptake contributes directly to environmental issues related to the overabundance of reactive N compounds which can become air and water pollutants.

Nitrate leaching is possible in either synthetic N-based or legume based systems. Leaching is a bigger problem in saturated soils that have high hydraulic conductivities or in soils that have been artificially drained and then receive high levels of irrigation or precipitation (Crews and Peoples, 2003). Nitrogen may leach in the form of nitrate any time there is a buildup of nitrates in the soil and water is supplied in excess of crop or forage needs, or with precipitation. Nitrate accumulation in the soil may be caused by nitrification of ammonium produced from mineralization of organic matter, or nitrate may accumulate due to N fertilizer application. Nitrate anions and base cations dissociate in water and both may leach through the soil profile, although the positive cations are more likely to be retained with the negative charges in the soil. These nitrates may also flow laterally if soil horizons are impermeable and may end up in ground water basins, lakes, rivers, and eventually travel into coastal and marine ecosystems (Schindler, 1978). Nitrogen leaching into aquatic or marine systems may be detrimental to water quality and lead to eutrophication and hypoxia which can in turn cause decreasing aquatic plant species diversity (Mosier et al., 2002). Nitrogen can leave an ecosystem in a variety of other ways as well. Nitrogen can leach as dissolved organic N, or in gaseous form as ammonia, N_2, nitrous oxide, or nitric oxide, all of which with the exception of N_2 can be linked to environmental hazards (Peoples et al., 1995).

Deposition of excess N in soil systems has led to acidification in some cases. This acidification can negatively influence crop and forest production systems by lowering soil pH (described by Mosier et al., 2002). However, the degree and rate of acidification depends on the form of N applied. Reduced inorganic N or ammonification of organic matter (e.g., legume residue) does not directly lower soil pH. Ammonium releases a hydrogen ion during the process of nitrification (Kennedy, 1992).

Losses of N from ammonia volatilization can also be substantial and may lead to increased rates of soil acidification. Exposure to ammonia gas can also result in greater vegetative sensitivity to drought or frost. There is an increased demand for carbon skeletons to assimilate NH_3 associated with ammonia gas exposure. This demand for carbon skeletons increases CO_2 uptake which in turn stimulates stomatal opening and water loss reducing drought tolerance. Also, growth of plant shoots is enhanced by NH_3 compared to root growth. Therefore, water supply from the roots may not be sufficient during drought (Fangmeier et al. 1994). As far as decreased frost resistance, low to moderate concentrations of NH_3 prolongs the growth phase in autumn and delays winter hardiness (Dueck et al. 1990). Ammonia volatilization can be reduced with the incorporation of soil amendments. Incorporating fertilizers and plant residues into the soil may also reduce the amount of N that can be lost from ammonia volatilization, but in doing so there is an increased risk for N loss through denitrification of the NO_3^- produced from nitrification (Peoples et al., 1995).

The emission of excess N into the tropospheric ozone layer contributes to its acidification (Galloway et al., 2002). Nitrous oxide and NO_x (NO and NO_2) are known to cause photo-oxidation of ozone. Ozone depletion, other oxidant deposition, and acid deposition associated with NO_x emissions can also damage manmade structures and cultural artifacts. Harmful emissions also have the potential to affect areas far away from their source. Molecules of reactive N have the ability to travel through aerial, aquatic, and terrestrial systems and have a compounding effect across all systems whether through acidification, hypoxia, or eutrophication.

N cycling in the environment

Two processes that contribute N inputs to terrestrial ecosystems are diazotrophy and lightning strikes. These processes change unavailable N into biologically available forms (Vitousek et al., 1997). Diazotrophy is the input of N into a biological system by the process of N_2 fixation typically through bacteria (Bagwell et al., 1998). There are many species of bacteria that have the ability to fix atmospheric N_2, some of which have symbiotic relationships with greater organisms. From the perspective of agriculture, *Rhizobium* bacteria are the most important. *Rhizobia* colonize root nodules of legumes (Bergersen, 1982) and fix atmospheric N_2.

Legumes

Some plants are able to obtain N for growth and development through their association with bacteria that fix atmospheric N_2 (Lindemann, 2003). Legumes are well known for their ability to fix N through the process of biological N_2fixation (Ball et al., 2007). The symbiotic relationship legumes and a few other dichotomous plants have with soil bacteria such as *Rhizobium* and *Frankia* makes this possible (Lindemann, 2003). *Rhizobium* bacteria live in nodules located at the roots of legumes (Bergersen, 1982). The host plant supplies the bacteria with oxygen and energy in form of carbohydrates, and in turn the bacteria provide the plant with ammonium. Annual legumes can produce approximately 87 to 111 kg N ha^{-1} $year^{-1}$ and perennial legumes can produce > 111 kg N ha^{-1} $year^{-1}$ in a cropping system (Lindemann, 2003).

Gettle et al. (1996) stated that legumes could be used in combination with switchgrass to provide N to the associated switchgrass, improve forage quality, and to extend the grazing season. Huneycutt et al. (1988) also conducted a study that compared responses of forages to broiler litter and fertilizer using tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire], bermudagrass [*Cynodon dactylon* (L.) Pers.], and mixed tall fescue-clover stands. They concluded that the greatest yielding unfertilized check plots occurred in the fescue-clover plots, and that low-cost high-yield stands of fescue-clover could be produced in absence of inorganic N fertilizer. Additionally, a large portion of the protein in legumes is in the form of biologically available protein or rumen degradable protein (Broderick, 1995). Legumes lead to increased N consumption and greater digestibility of ruminant diets. They can also increase food particle retention time, thereby increasing utilization and digestion of feedstuffs in ruminants (Foster et al., 2009).

General taxonomy of legumes

Plants that build root nodules with the filamentous bacterium *Frankia* are referred to as actinorhizal plants and are found in the Rosid clade throughout many different families. Closely related genera in the same family may or may not be nodulated. Plants that are nodulated with *rhizobia* reside only in the genera *Leguminosae* within the family *Fabales*. The one exception to this is the *Parasponia*. There are three subfamilies within *Fabales*: *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae*. The subfamilies are listed in order of the frequency of occurrence of nodulation with *Caesalpiniodieae* having the least frequency of nodules per plant and *Papilionoideae* having the greatest frequency of nodules per plant. The *Caesalpiniodieae* consist mainly of trees that grow in tropical rainforests, but may be less frequently found in temperate areas such as the grassy habitats of New England. The *Caesalpiniodieae* have fixation threads that contain the Rhizobia bacteria and allow them to move between cells in addition to their indeterminate nodule forms. The *Mimosoideae* plants that have the ability to nodulate do not exhibit fixation threads, but they share the same similar primitive nodule form of the *Caesalpiniodieae*. The *Mimosoideae* subfamily contains plants like the Acacia tree. The *Papilionoideae* subfamily is the largest and contains many legumes of agricultural interest such as white clover [*Trifolium repens* (L.)], red clover [*Trifolium pretense* (L.)], alfalfa [*Medicago sativa* (L.)], crimson clover [*Trifolium incarnatum* (L.)], hairy vetch [*Vicia villosa* (Roth)], and arrowleaf clover [*Trifolium vesiculosum* (Savi)] (Sprent, 2007).

Ecophysiology of legumes

Legumes are generally “cool season” C_3 plants which are less photosynthetically efficient than “warm season” C_4 plants at equal levels of solar radiation. The C_3 plants are also less drought resistant, heat tolerant, and have lower water-use efficiency than C_4 plants. However, cellular adaptations that help C_4 plants survive in warmer and drier climates also decrease their forage quality. Most C_4 grasses have a lower concentration of mesophyll cell volume per unit leaf volume than C_3 grasses. The protein content of C_3 mesophyll cells is also greater than that of C_4 cells (Nelson and Moser, 1994).

Some legumes such as alfalfa have a photosynthetic output that is between that of warm and cool-season grasses even though their enzymes and leaf anatomy are strictly C_3. Lespedeza is considered a warm-season legume and its photosynthetic output is about half that of alfalfa. A cool-season legume such as white clover has greater biomass production at lower temperatures than alfalfa. A cool-season legume's photosynthesis is similar to that of cool-season grasses (e.g., tall fescue) and greatly exceeds that of true warm-season legumes (e.g., peanut). Another difference between cool- and warm-season legumes is the transport of fixed N from the nodule to the plant. Cool-season legumes transfer N as amides, whereas warm-season legumes transport N in the form of ureides. This adaptation is thought to aid with heat tolerance in warm-season legumes through lowered respiration levels (Nelson and Moser, 1994).

Legumes contain large amounts of crude protein (CP) which typically range from 15-23% overall in aboveground biomass, although in leaves CP concentrations can exceed 25%. These high concentrations make legumes one of the most important sources of protein in animal diets. The fibrous components of legumes are mostly concentrated in the stem of the plant, making legume leaves highly digestible. Fiber components found in legume stems are slightly less digestible than grass fiber due to a greater level of legume fiber lignification. However, legume fiber is greater in overall nutritive value despite its lignification due to increased solubility (Vasiljević, 2009).

Biological N_2 fixation

The rate and extent of N_2 fixation is affected by many factors. Temperature, soil moisture, soil pH, *Rhizobium* population, soil fertility, leaf area, time of day, and certain minerals can all influence the amount of N_2 fixed. Although N_2fixation can be affected by many factors, there are three general categories of biological N_2 fixation limitations.

First, it is expensive in terms of energy for the plant to participate in N fixation. The plant must divert a large portion of energy that would be used for growth to the nodules in order for the symbiotic bacteria to have energy for fixation. This potential reduction in growth puts the legumes at a disadvantage for capturing solar radiation in comparison to non-leguminous plants. Therefore, N_2 fixation is usually reserved for environments that are flat, unshaded, open, and high in light. Leguminous trees found in dense forests generally have low levels of nodulation (Wedin and Russelle, 2007). An increase in leaf surface area and light exposure will increase photosynthesis which is positively correlated with N_2 fixation.

Biological N fixation's second major limitation is its dependence on high levels of elements including potassium, calcium, phosphorus, molybdenum, iron, and sulfur. A legume might have adequate sunlight to produce energy for N_2 fixation. However, if the soil it resides in is highly weathered with a low pH, secondary elements may not be readily available. Fertilizers containing these minerals are required in order for successful legume establishment and N_2 fixation. Molybdenum is an essential element in nitrogenase which is necessary for nitrogen fixation. Sulfur and Molybdenum are both present in nitrate reductase which is responsible for converting plant nitrogen into ammonium. Molybdenum and Iron are present in leghemoglobin which is found in the root nodules and is responsible for binding oxygen. The binding of oxygen creates a low oxygen atmosphere that allows the rhizobium bacteria to survive and fix nitrogen.

Insufficient levels of calcium and potassium can limit N_2 fixation. Calcium deficiency can negatively impact the transfer of N from the nodule to the rest of the plant, and potassium deficiency prevents the nodules from receiving adequate carbohydrate supplies for maximum N_2 fixation. Phosphorus promotes root nodule development in legumes by providing adequate energy (Wedin and Russelle, 2007).

Application of N fertilizer can negatively impact N_2 fixation. As a result, competition may increase from weeds and other plants whose growth will use up minerals such as potassium. As these competing plants flourish, they can also have a shading effect on legumes which in turn can reduce photosynthesis, thereby decreasing N_2 fixation (Ball et al., 2007).

The third factor that influences the success or failure of legume establishment and persistence as well as the ability to fix N_2 is herbivory. Low N availability in soils is reflected by low concentrations of CP in non-leguminous plants. Protein content makes legumes a preferred target for grazing. The reduction of herbivory of generalist herbivores (large ruminants) and specialist herbivores (many invertebrates) in a variety of ecosystems has increased the levels of N_2 fixation and overall legume production. Some legumes have even developed their own defense systems such as physical (spines) or chemical (bitter taste) adaptations to prevent herbivory (Wedin and Russelle, 2007).

Ecosystem functions of legumes

Soil organisms and plant roots play key roles in many ecosystem services including nutrient supply, water regulation, and maintenance of soil structure. The goal of using legumes in a grassland or pasture setting is to eliminate or at least greatly reduce the requirement for N fertilizer, but there is still much research required before the effects that legumes have on soil biota and functioning of the plant-soil system are fully understood (Eekeren et al., 2009). For instance, white clover root mass is lower than that of grass which could decrease available food sources for soil biota and in turn decrease microbial productivity.

Decreased activity of soil biota can decrease soil structure and integrity. In fact, when soil aggregates from fields growing white clover were compared to fields growing perennial ryegrass there were a lower percentage of stable aggregates from the white clover (Robinson and Jacques, 1958). While legume-grass mixtures are beneficial theoretically, it is important to empirically study their effects to understand impacts at a systems level.

Legumes, like fertilizer, have potential inefficiencies related to N. The biggest potential loss from legume-based systems may occur during summer or during winter fallows after residue incorporation but before the planting of the subsequent crop or forage. If leguminous cover crops are cultivated during the fallow season, they can retain N in the terrestrial system because they will scavenge available soil N in addition to N they fix. This may reduce N losses from leaching (Crews and Peoples, 2003).There is also evidence that N leaching can be reduced in soils covered with legumes compared to the amount of N that may be potentially leached when using fertilizers. A 15-year study by Drinkwater et al. (1998) measured the amount of leached nitrate from legume-based, manure-based, and fertilizer-based cropping systems for maize. These authors found that legume and manure-based systems both averaged losses of approximately 13 kg NO_3-N ha^{-1} and that the tested fertilizer-based system averaged a nitrate loss of approximately 20 kg NO_3-N ha^{-1}. Studies have also shown a decrease in leaching under grass-legume mixed swards compared with fertilized grass. Owens et al. (1994) measured nitrate leaching in several perennial pastures using lysimeters. In their study, nitrogen was supplied by fertilizer for 5 years and alfalfa was inter-seeded into orchardgrass and tall fescue.

Nitrate leaching was reduced between 48 and 76% after the N source was switched from fertilizer to alfalfa. However, according to Crews and Peoples (2003), it is important to note that the results of studies that try to determine N efficiency, whether for cropping or pasture systems, depend on whether best-management N fertilizer practices were used, the rate of fertilizer that was applied, the legume content of the pastures, and even whether the legume and grass species were annuals or perennials.

Legumes have a tendency to absorb high amounts of soil base cations, and in the process of balancing their internal charge, discharge H^+ into the rhizosphere. This H^+ deposition may cause soil acidification after the legumes are harvested or grazed. However, if legume biomass is reincorporated into the soil as with some green manure practices, there is no net soil acidification (Crews and Peoples, 2003). When legumes are reincorporated into the soil there is also less chance that nutrients will escape via the gaseous state.

A study by Robertson et al. (2000) lasting over 9 years addressed greenhouse gas output associated with varying cropping production systems that were either fertilizer- or legume-based. Each greenhouse gas associated with production (CO_2, N_2O, and CH_4 in particular) was evaluated based on its potential danger as a greenhouse gas and then assigned accordingly into a global warming potential index (GWP). The study revealed that the conventionally tilled and fertilized production system had a net GWP of 114, the legume-based tilled cropping system had a GWP of 41, and the no-till fertilized system had an initial GWP of 14 before the carbon sequestered in the soil was released at a later time. The substantially greater GWP of the conventionally tilled and fertilized system can be largely attributed to the amount of fossil fuel required to produce the N fertilizer and to apply lime. Initially, the same amount of fossil fuel is also used in the no-till fertilized system to produce N fertilizer and apply it including the lime, but the C already present in the soil is trapped for a period of time lowering the initial GWP. However, the authors predicted once equilibrium in soil organic matter is reached in the no-till fertilized soil, the GWP will climb to a point that it is closely comparable to the conventionally fertilized and tilled system. Thus, ultimately the legume-based tilled system contributes the least to global warming.

Forage legumes and their agricultural importance

Forage legumes can be used in animal rations as pasture, hay, or silage. When used in a pasture setting, legumes may improve the soil by increasing soil organic matter, improving soil porosity, recycling nutrients, improving soil structure, decreasing soil pH, and diversifying the microscopic life in the soil. Legumes also offer soil conservation properties, provide N for other grasses that do not have the ability to fix N through above and below ground legume decomposition, and produce flowers which ultimately result in the production of honey (Sheaffer and Evers, 2007). However, it is important to note that there are still difficulties associated with legume utilization. According to Rochon et al. (2004), legumes can be difficult to establish and long-term persistence is difficult to maintain without intensive management practices. Additionally, varying geographic and climatic conditions make it difficult to select a well-adapted high-yielding species of legume for each production setting. Each species of legume is adapted to different temperatures, duration of daylight, soil moisture content, soil pH, and availability of soil nutrients (Whyte, 1953). Problems associated with legume establishment and persistence make it difficult for farm operators to entirely forgo conventional N fertilizer and integrate legumes into their cropping or pasture rotations.

Legume establishment

Establishing the desired legume-grass mixture is difficult because the grass and legume components respond differently to environmental factors (Blaser et al., 1956). There is a greater resistance for an additional species to establish when there are already a large number of different plants present in a target sward. New species that are able to establish despite resistance usually fill a specific niche (Harmoney et al., 2001). However, there are methods to increase a legume’s chances for establishment. Taylor and Allinson (1983) were able to successfully establish legumes into a grass sod by disturbing the forage canopy with only three evenly spaced clipping periods instead of disking the ground or using chemicals to weaken the canopy. Another simple and low-cost establishment method that has been successful in the past is frost-seeding. Frost seeding is broadcasting forage seed onto the ground surface while the ground is still frozen in the spring. The principle is that repeated freezing and thawing of the soil surface causes surface cracks in the soil which allow seed incorporation. This method has been successful when seeding legumes into cool-season grass in the U.S. Midwest and also into warm-season grasses (Gettle et al., 1996). Frost-seeding has the greatest potential success when the surface of the soil is covered with ice crystal honeycombs. When the soil cracks and shifts from freezing, thawing, and rainfall, there is generally enough seed-to-soil contact (Gettle et al., 1996).

Legume persistence

A common concern with using legumes in pastures is legume persistence. Persistence problems arise from environmental stress including grazing (Harmoney et al., 2001). The ratio of grass-to-legume species may increase yearly which may potentially cause a need for N fertilizer to maintain forage production at an acceptable level (Gettle et al., 1996). However, species including red clover, alfalfa, and birdsfoot trefoil can dominate other grass species in certain environmental situations (Gettle et al., 1996).

Perennials: Alfalfa originated in Iran and central Asia, and it has been grown for almost 9,000 years (Ball et al., 2007). Alfalfa is thus the oldest crop grown for the sole purpose of forage. It now occupies approximately 10 million ha in the United States, making it the predominant perennial legume species. About 25% of its production is accounted for in the western U.S., and another 50% is in the north central region of the U.S. which includes North Dakota, Wisconsin, and Minnesota. Alfalfa can have 2 to 10 regrowth cycles depending on maturity at harvest and the length of the growing season (Sheaffer and Evers, 2007). To establish an alfalfa stand, a firm seedbed is considered important. Alfalfa grows best in well-drained soils that are high in fertility and have neutral pH. A pH of 6.5 or greater is necessary for high yield (Ball et al., 2007). Alfalfa does not tolerate flooding or wet, saline soils. Cold tolerance is a major factor of success in the northern regions. Some cultivars can survive winter temperatures of -25^{o}C.

If managed properly, yield and forage quality of alfalfa hay are greatly influenced by its maturity at harvest. Frequent harvesting at the bud stage can increase forage quality but at the same time reduce yield and persistence (Sheaffer and Evers, 2007). To maintain persistence while keeping forage quality at acceptable levels, it is recommended to harvest at early flowering (Sheaffer and Evers, 2007; Ball et al., 2007). A stand will often persist for 3 to 5 years, but if good fertilizer practices are used and plants are cut at the proper growth stage, stands can persist for 8 years or longer (Ball et al., 2007).

In a grazing situation, it is also recommended to use alfalfa in mixed swards to reduce the likelihood of bloat (Sheaffer and Evers, 2007). Alfalfa can be highly digestible and can ferment more rapidly than many other types of forage. The rapid fermentation can produce large amounts of gas quickly that combine with digesta forming stable foam which has the ability to cause frothy bloat. Grazing-tolerant cultivars can be continuously stocked, but weed control and yields are typically better when stands are rotationally grazed with a 20 to 35-day rest period (Ball et al., 2007).

White clover originated in the Mediterranean region (Ball et al., 2007). Approximately half of the 45 million ha of humid or irrigated pasture in the U. S. contains white clover. It has a shallow root system, and the original tap root usually does not live very long. The plant compensates for this by indeterminate stolon growth. There are three main types of white clover based on morphological characteristics. The small type grows closer to the ground and is persistent under grazing, but produces little forage yield. The intermediate type falls between the small and large type and persists well because of high seed production. The large type produces very large petioles, peduncles, leaflets, flowers, and stolons making it the highest-yielding type. However, the fact that large-type white clover grows taller in conjunction with low flower production makes it prone to persistence problems under grazing. White clover has very poor heat and drought tolerance, and it does not grow well in sandy soils (Sheaffer and Evers, 2007). White clover is also more tolerant to wetter and more acidic soils. It also performs well when grown in conjunction with cool season perennial grasses (Ball et al., 2007). In the southern U.S., it is necessary to use high seed producing intermediate types in order to overcome persistence issues with heat and drought (Sheaffer and Evers, 2007). When grown in conjunction with grasses, it is important to ensure that grasses are not under-grazed to prevent them from competing with the clover (Ball et al., 2007). White clover is typically used as forage. Its growth habits make it suitable for continuous grazing, but it will perform better in terms of yield and persistence if rotationally grazed with a rest period (Sheaffer and Evers, 2007). White clover is also known to cause bloat (Ball et al., 2007).

Red clover originated in Southeastern Europe and Turkey (Ball et al., 2007). There are three main types of red clover. The most common in the U. S. is the medium type grown in the northern region. It is an early flowering type that can produce 2 to 4 hay crops per growing season and is typically biennial. The mammoth type is later flowering and typically only produces one cutting with an aftermath. The mammoth type does not usually flower in the seedling year. The third type of red clover is a wild variety found in England. The medium type grown in the U. S. covers about 4 million ha and is most common in the north central and northeastern regions. Red clover grows best in environments where summer temperatures are mild and sufficient soil moisture is present. In comparison to alfalfa, red clover is less heat and drought tolerant, but it has the ability to thrive in more diverse soil conditions. It can grow in soil pH as low as 5.5 making it a good alternative to alfalfa in low pH soils (Sheaffer and Evers, 2007). It can also grow in soils that have poor drainage, but it is less tolerant than white clover in extremely wet conditions (Ball et al., 2007). Red clover results in similar animal performance as alfalfa, although it is typically shorter-lived than alfalfa due to lack of disease resistance and winter hardiness (Sheaffer and Evers, 2007). Red clover may also provide more grazing than white clover in the summer, but it will not tolerate continuous grazing like white clover (Ball et al., 2007). Persistence of red clover can be greatly increased with rotational grazing (Ball et al., 2007 and Sheaffer and Evers, 2007). Seedlings are especially competitive making establishment easier (Sheaffer and Evers, 2007).

Annuals: Crimson clover originated from the Mediterranean region (Ball et al., 2007). In the U. S., it is grown in the southeastern region for grazing purposes and sometimes for hay production. Crimson clover establishes rapidly due to its high seedling vigor. It also grows on varied soil types including sandy soils and well drained clays, and it tolerates pH values from 5 to 7. Crimson clover is very cold tolerant as well. At temperatures as low as 5.9^{o}C, it can be the most productive of the different cool season clover varieties. Crimson clover's high seedling vigor and early maturity make it an excellent clover to interseed into warm season grasses like bermudagrass (Sheaffer and Evers, 2007). It can be grazed throughout the winter, but in order to produce a hay crop, cattle must be taken off pasture by mid-March (Ball et al., 2007).

Arrowleaf clover is another species that originated from the Mediterranean region (Ball et al., 2007). In the U. S., arrowleaf clover is cultivated with success in the mid-south region, particularly in Oklahoma and Texas. However, more recently, use has declined because of root rot disease and viruses. Arrowleaf clover produces approximately 90% hard seed making it exceptional in terms of reseeding. It performs the best in well drained loamy or sandy soils and does not tolerate clay or poorly drained soils well. Its optimal pH is about 6.5 (Sheaffer and Evers, 2007). Seedling vigor and growth are low; therefore, there is usually little forage production until early March. Overall it is one of the latest maturing of all clovers, but it is highly productive. If moisture is plentiful, it can continue to grow through June and July. Arrowleaf clover can be grazed or used for hay purposes (Sheaffer and Evers, 2007). However, after hay cutting regrowth may not occur (Ball et al., 2007). Its forage quality is superior to that of crimson clover at all times during the growing season (Ball et al., 2007). It also has high tannin levels, making bloat risk a nonentity (Sheaffer and Evers, 2007).

Hairy vetch also originated in the Mediterranean region (Ball et al., 2007). Hairy vetch and common vetch are the two most utilized vetches in the U. S. and are cultivated in most regions throughout the country. Hairy vetch can grow in all soil types provided they are well drained, and it is one of the more acid-tolerant of the forage legumes. Hairy vetch is also resistant to cold temperatures (Sheaffer and Evers, 2007). Vetch is usually grown as a winter cover plant, forage, or for green manure. Grazing should not begin until the plants are at least 15 cm tall. Animals should be moved off of the vetch plants before the lowest leaf axil is grazed (Sheaffer and Evers, 2007).

Forage legumes in grazing systems

Perhaps one of the most important factors for using legumes in pasture systems is the legumes' impact on animal performance. Not only do legumes typically need little to no N fertilizer, but they can also extend the grazing season (Gettle et al., 1996). Lomas et al. (2004) reported that legumes improved the nutritive quality of pastures and increased gains of grazing livestock. It is possible for animals grazing legumes to grow faster and have better productivity per unit of land area than animals grazing grass-only pastures (Mouriño et al., 2003). Burns and Standaert (1985) stated that cattle average daily gain and gain per hectare are usually greater on legume-grass systems until N application rates on N-grass systems exceed 200 kg ha^{-1}. In Australia, tropical legume-based pastures mesh well with beef finishing operations (Hill et al., 2009). The legume-based pastures provide larger cattle at a younger age due to high annual growth rate. This decreased period of grazing for cattle also provides improved market opportunities (Hill et al., 2009). In another study, legume-grass mixed pastures showed an animal liveweight gain of 120 kg head^{-1}year^{-1}in the first year of establishment, but in the four years following the first, animal liveweight gain improved ranging from 160 to 200 kg $head^{-1}year^{-1}$ (Clem, 2004). Mouriño et al. (2003) reported markedly improved steer performance on pastures that contained a mixed stand of kura clover and grass. As little as 6 % alfalfa in a stand of endophyte infected fescue was able to improve average daily gain and somewhat offset the negative effects of fescue toxicosis (Hoveland et al., 1997). The increase in animal performance on legume pastures when compared to grass pastures can be linked to the greater protein content and digestibility of the legumes (Bhatti et al., 2007 and Hill et al., 2009). A pasture that contains legumes in conjunction with grass has the ability to provide more protein for a longer period of time. This prolonged protein cache helps legume-grass mixed pastures to outperform grass-only pastures in terms of animal production (Coates et al., 1997).

One of the more common problems associated with legumes is bloat. Bloat is caused by highly digestible, rapidly fermentable diets. The gas from the substrate mixes with digesta in the rumen and forms stable foam. This foam traps gases in the rumen preventing eructation and hindering breathing. According to Lauriault et al. (2005), hungry animals that are turned into a pasture that contains a fresh stand of legumes are more likely to get bloat. Some animals also appear to be more susceptible to bloat than others due to their genetics or species. However, there are certain management practices that will reduce the likelihood of bloat. In order to keep animals from rapidly grazing lush legumes, they should be rotated to leguminous pastures after a period of morning grazing on the preceding pasture. Certain supplements can also be provided to aid in preventing bloat such as poloxalene (Lauriault et al., 2005).

Summary and objectives of research

Applying N fertilizer is a low-risk and well-established method for increasing forage yields. However, the cost of N fertilizer increases whenever fossil fuel prices increase. Also, N fertilizer applications can result in negative environmental effects under certain circumstances. A substitute may be necessary in order to alleviate economic and environmental problems in the future.

Legumes may be viable substitutes when compared to conventional N fertilizer schemes. They have the potential to increase forage quality, increase the length of the grazing season, and increase animal performance. However, legumes are not without disadvantages. Legumes are often difficult to establish and also have persistence problems because of their lack of heat tolerance and reliance on abundant moisture. This is especially true in the southern U. S. where summers are often hot and dry. Therefore, the objective of this research was to identify suitable annual and perennial legume species for utilization in grazing systems by:

- Evaluating effects on animal performance and soil quality of selected legume species,
- Evaluating persistence of various legume species, and
- Evaluating forage mass production of various legume species

Two experiments were conducted at two different locations in order to achieve these objectives. Experiment 1 was conducted to evaluate animal performance, forage mass production, and soil quality associated with legumes over-seeded with ryegrass into bermudagrass pastures. Experiment 2 was conducted to evaluate the persistence of six legume species over-seeded into existing bermudagrass pastures.

Materials and Methods

Experiment 1: Monticello

Region/Landscape

This research was conducted between 2008 and 2010 on experimental pastures at the University of Arkansas Southeast Research and Extension Center (SEREC) in Monticello, AR located in Drew County (91°48'W; 33°35'N). According to the USDA-Natural Resources Conservation Service (Accessed March 6, 2011), soils at the research site were an Amy Silt loam (fine-silty, siliceous, semiactive, thermic Typic Endoaquults), a Sacul (fine, mixed, active, thermic Aquic Hapludults), and a Tippah silt loam (fine-silty, mixed, active, thermic Aquic Paleudalfs), and the landscape featured slopes of up to 3%. The area has an annual mean precipitation of approximately 1407 mm and an average air temperature of 16.7°C (United States Climate Data, Accessed March 6, 2011).

Clover and Ryegrass Establishment

The experimental pastures consisted of established common bermudagrass and were randomly selected for overseeding with ryegrass [*Lolium multiflorum* (L). cv. 'Marshall'] and either crimson clover (C; cv. 'Dixie'), white clover (L; cv. 'Osceola'), or both (CL). Prior to broadcasting, pastures were disked lightly. Pastures were also dragged using a 3-m chain harrow in order to smooth the surface and improve soil-to-seed contact. Seeding rates were 34 kg ha^{-1} (actual seeding rate), 11 kg ha^{-1} (pure live seed; PLS), and 5 kg ha^{-1} (PLS) for ryegrass, crimson clover, and white clover, respectively. White clover seeds contained a coating; hence product seeding rate was 7 kg ha^{-1} PLS. Seeding rates for the white clover and crimson clover in the CL pasture remained the same as in C and L pastures in order for each species to fully represent its respective grazing period. The remaining 2 control pastures (Con) were overseeded with annual ryegrass only. Pastures were re-seeded every year.

Fertilizer Applications

A timeline that displays the activities between November 2008 and August 2010 during this study is presented in Fig. 1. On November 3, 2008, Con treatments received 336 kg ha^{-1} of 19-19-19 (64 kg ha^{-1} actual N). The following day, November 4, 2008, legume treatments received 224 kg ha^{-1} of 0-23-30. On February 23, 2009, Con treatments received 168 kg ha^{-1} of 34-0-0 (57 kg ha^{-1} actual N) as spring application. The same day, pastures with legumes also received ammonium nitrate in the amount of 67 kg ha^{-1} (23 kg ha^{-1} actual N). It was recognized at this point that without early-spring fertilization of legume treatments, initiation of grazing in these pastures and therefore grazing days among treatments would likely differ to an extent which would make treatment comparisons unrealistic. The Con treatments were fertilized again June 3, 2009 with 168 kg ha^{-1} of 34-0-0 (57 kg ha^{-1} actual N). The fertilizer quantities applied were considered large enough to initiate ryegrass biomass production yet small enough to not limit N fixation rates substantially.

On November 9, 2009, Con treatments received 336 kg ha^{-1} of 19-19-19 (64 kg ha-1 actual N). Legume treatments received 336 kg ha^{-1} of 6-24-24 (20 kg ha^{-1} actual N) the same day. On March 4, 2010, Con treatments received 202 kg ha^{-1} ammonium nitrate (68 kg ha^{-1} actual N), and legume treatments received 112 kg ha^{-1} ammonium nitrate (38 kg ha^{-1} actual N). A quantity of 3.36 Mg/ha of lime was also applied in 2009.

Biomass Production/Removal

Forage mass was determined using a calibrated disk meter taking 10 measurements before cattle were stocked and after cattle were removed from each grazing cell. Five clippings were then taken at random using hand shears and a 0.25-m^2 quadrat. These samples were placed in paper bags and transferred to a forced-air oven and dried at 50°C until no further weight loss was detected. Regression analysis was used to determine a quadratic equation to correlate the harvested forage mass from the five random sample sites to the falling plate meter readings obtained from each cell. Forage removal was calculated by subtracting the forage mass calculated from the regression equation after cattle were removed from forage mass calculated from the regression equation before cattle were stocked and then adding a correction factor for forage growth during the grazing period. The correction factor for forage growth was determined by taking forage mass measurements in the opposing cell of the same pasture that cattle were not grazing in during the same period. Forage mass calculated from the regression equation at the beginning of the grazing period for the ungrazed cell was subtracted from the final forage mass calculated from the regression equation at the end of the grazing period for the ungrazed cell.

Cattle Management

Gelbvieh × Angus crossbred spring-born heifers [n = 40; 242.88 ± 9.71 kg initial body weight (BW)] from the University of Arkansas Livestock and Forestry Research Station near Batesville, AR were used for this study and shipped to the Southeast Research and Extension Center (SEREC) in Monticello, AR. Heifers remained as a group upon arrival at SEREC and were placed on a dormant common bermudagrass pasture and given bermudagrass hay ad libitum. The groups were then stratified by BW and assigned randomly to one of the eight, 2-ha pastures which were divided into two equally sized grazing cells using temporary electric fencing. Animals were rotated between cells every 14 d after initiation of grazing and weighed every 28 days.

Animals were moved onto pastures at the beginning of spring and summer when clovers reached a height of at least 7.5 cm on average. During the first year of the study, cattle were placed on Con pastures January 23, 2009. However, grazing on legume treatment pastures was not initiated until March 6, 2009 due to a lack of available forage. Heifers remained on their respective pastures until May 11, 2009. Remaining forage biomass was cut for hay on May 27, 2009. Beefmaster or Beefmaster × Angus crossbred heifers and steers from the SEREC herd (n = 64; 292.94 ± 18.93 kg initial BW) were added to the pastures on June 22, 2009 for the summer grazing. Pastures and rotations were managed the same as in spring. Cattle were removed from the pastures August 27, 2009.

For the second year of study, the randomization structure of treatments remained. Heifers were again rotated between cells and weighed at the same intervals used in the first year. Heifers from Batesville were stocked on their respective pastures on March 15, 2010 when forage biomass was great enough to begin grazing. In 2010, grazing days were possibly affected across all pastures due to heavy damage by grazing wildlife early in 2010. Cattle remained on their respective pastures until May 12, 2010. The remaining forage biomass was cut for hay May 19, 2010. Beefmaster or Beefmaster × Angus crossbred heifers and steers from the SEREC herd were added to the pastures on July 7, 2010 for the summer portion of the trial. The pastures and rotations were managed the same in summer as they were in spring. Cattle were removed from the pastures August 31, 2010. Grazing activities are also displayed in the timeline of Figure 7.

Experiment 2: Batesville

Region/Landscape

This experiment was conducted between 2008 and 2010 at the University of Arkansas Southeast Livestock and Forestry Research Station in Batesville, AR located in Independence County (91°46'W; 35°49'N). Soils at the research site were a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) and a Noark very cherty silt loam (clayey-skeletal, mixed, semiactive, mesic Typic Paleudults). The area has an annual mean precipitation of approximately 1,222 mm and an annual mean air temperature of 14.7°C (United States Climate Data, Accessed March 6, 2011).

Legume Establishment

The experiment was designed as a randomized complete block with six replications for each legume species. Each block contained one control paddock which was not altered in terms of species composition. The 2-ha experimental pastures consisted of common bermudagrass and were randomly selected for overseeding with either perennial or annual legumes. Before initial planting of legume species, Roundup (The Scotts Company LLC, Marysville, Ohio) was applied at approximately 1.2 L ha^{-1}to repress bermudagrass growth. Perennial legumes used were white clover (cv. 'Durana'), alfalfa (cv. 'Ameristand' 403T), and red clover (cv. 'Cinnamon Plus'). Annual legume species were crimson clover (cv. 'Dixie'), hairy vetch (cv. VNS), and arrowleaf clover (cv. 'Yucchi'). A John Deere 5425 tractor (75 hp; John Deere, Moline, Illinois) and a Haybuster model 107 no-till drill (Haybuster Agricultural Products, Jamestown, ND) were used for seeding October 5, 2007. Seeding rates were approximately 6 kg ha^{-1} for white clover, 27 kg ha^{-1} for alfalfa, 17 kg ha^{-1} for red clover, 38 kg ha^{-1} for crimson clover, 32 kg ha^{-1} for hairy vetch, and 13 kg ha^{-1} for arrowleaf clover. For an overview, seeding rates are displayed in Table 18. White clover, alfalfa, and red clover seeds contained a coating and thus were seeded at rates accounting for the coating to maintain recommended densities. On November 16, 2007, Post Plus (BASF Corporation, Research Triangle Park, North Carolina) was applied at approximately 1.75 L ha^{-1}, and Select (Valent U.S.A., Walnut Creek, California) was applied at approximately 0.6 L ha^{-1}. Both were mixed with approximately 2.3L ha^{-1}crop oil. Potash (112 kg ha^{-1}, 0-0-60) was applied in September 2008. Lime was also applied at 4.48 Mg/ha in 2008. Mustang Max was applied at a rate of 0.1 L/ha in April, June, and August of 2008 to control weevils in alfalfa plots.

Pasture Management

Six groups of cattle were used to graze all blocks for perennial and annual treatments at the same time (total of 6 pastures containing 2 blocks each for perennial and annual species). Gelbvieh x Angus crossbred calves were stocked at an average rate of 1250 kg/ha in each block once canopy height was at least 27 cm. Animals were separated into groups with similar weight and moved to their respective pasture where they remained for approximately 5-7 days. Canopy height at the end of each grazing cycle was approximately 7.5 cm. Rest periods between grazing cycles were 28 days on average. Pastures and blocks were grazed simultaneously during the entire duration of the experiment with one exception: During spring of 2010, the number of animals at the research station available for grazing was not sufficient to stock all pastures at the same time. Therefore, three groups of cattle grazed pastures sequentially for two cycles. During July of the same year, grazing schemes returned to the same schedule, i.e., all six pastures were grazed by six groups simultaneously for each cycle until the end of the growing season. During the first year, rotations began April 18, 2008 and continued until October 21, 2008. In the second year, rotations began April 1, 2009 and continued until September 28, 2009. During the third and final year of study, rotation began April 12, 2010 and ended September 14, 2010.

Above-ground Biomass Production

Forage aboveground biomass production was measured by taking clippings with a gas-engine hedge trimmer (Poulan, Charlotte, NC) from a 0.25-m^2 quadrat each time the cattle either entered or exited a pasture. In each plot, 4 quadrats of forage were clipped and transferred to paper bags and weighed in the field. A sub-sample was taken from each of the 4 samples and placed in a separate paper bag as a composite sample for each plot which was transferred to a forced-air oven and dried at 50°C until no further weight loss was detected. This composite bag was then used to determine DM and also later for forage chemical composition analysis. The DM of this bag was determined by dividing the dry sample weight by the original wet weight in the field and multiplying by 100. This DM percentage of the composite bag was then used in order to determine the DM of the 4 original quadrats of forage clipped for each plot by multiplying the DM percentage by the weight of the 4 bags when they were originally weighed in the field.

Procedures Common to Experiment 1 and 2

Plant Species Composition

Forage species composition in experiment 1 and 2 was assessed using the wire frame method described by Vogel and Masters (2001). Frames used for both studies were obtained from used wire fence panels and contained 36, 15 x 15-cm^2 squares. In the process of assessing species composition, only photosynthetic plant material was counted. Species that were not identified as bermudagrass, ryegrass, or the appropriate legume species for the treatment being assessed were counted for category “other” (OP).

Plant Chemical Composition

For experiment 1, forage grab samples were obtained whenever cattle were moved to a new grazing cell. In experiment 2, subsamples were obtained from forage mass samples each time a new stocking cycle began. Similar for both experiments, samples were then placed into paper bags which were dried at 50°C in a forced-air oven until no further weight loss was detected. Samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen and analyzed for neutral-detergent fiber (NDF; ANKOM Technology Corp., Fairport, NY; Vogel et al., 1999), total N (AOAC, procedure 990.03), acid-detergent fiber (ADF; ANKOM Technology Corp., Fairport, NY; Vogel et al., 1999), acid-detergent lignin (ADL; Van Soest et al., 1991), and acid-detergent insoluble N (ADIN; Licitra et al., 1996). Ash content was assessed by heating dried samples at 500°C in a muffle furnace for 6 hours. The samples were then allowed to cool for at least 8 hours in the furnace before removing them and placing them in a dessicator for 45 minutes before weight determination. The new ash weight was then divided by the original dry sample and multiplied by 100 to determine percent ash.

Soil Quality Analysis

Soil samples were collected to a depth of 10 cm using 2-cm diameter stainless steel probes attached to a sliding hammer (Art's Manufacturing and Supply, Inc., American Falls, ID). Sixteen subsamples were taken randomly and composited in each pasture in the spring, summer, and fall of each year. All probes were sterilized before use and a different probe was used for each treatment. Samples were kept moist and put on ice in the field and sieved upon return to the lab through a 2-mm sieve.

To obtain gravimetric soil moisture, soil was dried at 105°C until a constant weight was achieved. Gravimetric soil moisture was calculated by subtracting the mass of the dry soil from the moist soil and dividing the difference by the dry soil mass. Organic matter was measured by loss-on-ignition. The soil dried at 105°C was ashed in a muffle furnace at 375°C for 1hr and followed by 550°C for 6 hr. The muffle furnace was allowed to cool to 50°C at which time the samples were removed, placed in a desiccator, allowed to cool to room temperature and weighed (Karam, 1993). The percentage of OM was calculated by subtracting the ash from the dry soil mass, dividing the difference by the dry soil mass, and multiplying by 100.

Microbial biomass (µg C g^{-1} soil) was determined using chloroform-fumigation-extraction (Vance et al., 1987). Fumigated soil was incubated for 24 hours in dark at 25^{o} C with ethanol-free chloroform. Fumigated and unfumigated soil (8 g) was extracted with 40 ml of 0.5 M K_2SO_4 with a 1:5 (wt:vol) soil-to-extractant ratio. The concentration of carbon in the extracts was determined with a Shimadzu TOC-V PC-controlled total organic carbon analyzer (Shimadzu, Columbia, MD). An aliquot of the fumigated and unfumigated extracts was oxidized to convert all of the N forms to nitrate (NO_3^-) by persulfate oxidation (Cabrera and Beare, 1993). Unfumigated, unoxidized extracts were analyzed colorimetrically on a Skalar segmented-flow autoanalyzer (Skalar Inc, Norcross, GA) for inorganic N (Ni) which consisted of ammonium-N (NH_4^+-N) plus NO_3^-—N. Ammonium-N was determined by the salicylate hypochlorite procedure and NO_3^--N was determined by the modified Griess-Ilosvay procedure (Mulvaney, 1996). Fumigated, oxidized extracts on the Skalar produced results for dissolved total N (DTN) (Jones and Willett, 2006). Dissolved organic N was calculated from the value of total dissolved N (TDN) minus Ni. Microbial biomass C and N were calculated as the difference in respective concentrations between fumigated and unfumigated samples and reported without a correction factor.

Dehydrogenase activities were determined on moist soil using the procedure outlined by Casida et al. (1964). Enzymatic activity including β-glucosaminidase was measured using procedures described by Parham and Deng (2000); β-Glucosidase enzyme activities were measured using Tabatabai's procedure (1994).

Statistical Procedures

Data collected from Experiment 1were analyzed using PROC MIXED of SAS. Treatment (C, CL, Con, or L) was the only factor that was included in the model when calf data, forage production data, and species composition data were processed. The terms 'year' and 'pasture within treatment' were included in the random statement. Data were analyzed by season as different animal groups were used for grazing in spring and summer.

Forage mass data from each separate collection date showed high variability across the different sampling dates which made an accurate calculation of forage removal and forage growth for each grazing cycle impossible. Therefore, forage mass was averaged across sampling dates for each season, using forage mass data that were collected whenever animals were placed into a new grazing cell.

Treatment effects on forage chemical composition were analyzed by including month in the model to test for a possible treatment x sampling date interaction within each season. The

LSMEANS with the PDIFF option was used for mean separation of treatment effects. Differences among means were considered significant at $P < 0.05$.

For Experiment 2, forage production, species composition, and chemical composition were also analyzed using the PROC MIXED procedure of SAS. Treatment, year, and month were included in the model. ‘Block’ and ‘treatment by block’ terms were included in the random statement. The LSMEANS statement including the PDIFF option was used for mean separations. Annual and perennial species were analyzed separately due to their differences in growth habit and life cycle. Differences described between means were considered significant at $P < 0.05$, unless otherwise noted.

Soil data for both experiments were analyzed using the PROC MIXED procedure of SAS. ‘Treatment’ and ‘season,’ and the ‘treatment by season’ interaction term (spring, summer, autumn) were included in the model. ‘Replication within treatment by year’, and ‘season by replication within treatment by year’ were included in the random statement. The LSMEANS statement with the PDIFF option was used to perform mean separations.

Results and Discussion

Experiment 1

Animal Performance and Forage Mass Production

Initial weight, end weight, and average daily gain (ADG) did not differ ($P > 0.05$) across treatments for the spring grazing season (Table 1). However, total gain was greater ($P < 0.05$) for Con compared with treatments C, L, and CL, but grazing days were also greater ($P < 0.05$) for Con compared to C, L, and CL during spring. During summer, none of the sampled animal performance indicators were affected by the treatments.

With regard to forage mass, forage removal by cattle, and total forage mass production over the course of the season there was no difference ($P > 0.05$) among treatments during spring (Table 2). For the summer season however, forage mass was greater ($P < 0.05$) from Con compared with CL. Forage mass from C and L was intermediate and did not differ ($P > 0.05$) from CL or Con. Forage removal or total forage production in summer did not differ ($P > 0.05$) among treatments.

The increased number of grazing days under the control treatment during spring resulted from uneven forage growth in the experimental pastures early during the study. During the initial year of the experiment, forage mass in the control treatment was 1,761 kg/ha compared with an average of 1,170 kg/ha for CL, C, and L (data not shown). Although those differences were somewhat mitigated during subsequent years through light N fertilization in legume treatments as described in the experimental methods, forage mass still appeared higher under Con with 2,156 kg/ha vs. an average of 1,563 kg/ha for the legume treatments. Clearly, the lack of forage growth in C, L, and CL was due to the absence of N fertilization such as that applied to the ryegrass in the control treatment at planting in fall.

Despite no observed differences, clovers may have contributed somewhat to forage mass production and nutritive value in an otherwise warm-season grass sward. Previous research has shown that grass-legume mixes can be as beneficial in terms of animal performance as N-fertilized pastures. For example, Mouriño et al. (2003) reported that animals grazing legumes grew faster and more efficiently per unit of land area. Burns and Standaert (1985) stated that ADG and gain per hectare are usually greater on legume-grass systems until N application rates on N-grass systems exceed 200 kg ha^{-1}. In the present study however, nitrogen fertilizer rates on Con pastures ranged from 168 kg ha^{-1} in spring and summer to 336 kg ha^{-1} in the fall, very likely offsetting any contributions in forage growth derived from recycled clover N in the legume treatments. Noted should be the higher forage mass under Con during summer. Being different from CL, this suggested that N turnover from clover was not sufficient to match the increased forage production when fertilized synthetically. High N turnover can only be achieved with high-yielding clovers and proper nutrient cycling because 95% of the N fixed is located in the above-ground biomass. It is possible that crimson clover is to be preferred over white clover due to the more upright growth and higher forage mass production than white clover that can rely on prostrate growth to survive. Based on the experience with this study, more accurate yet quick methods of forage mass determination need to be developed. Pasture species separation in the laboratory is highly accurate to determine the forage mass for each species but could not be performed during this study. Also, the stocking rate in our study could have been increased theoretically in order to determine if Con could have supported more cattle than the legume treatments. In summer, Con treatment had greater ($P < 0.05$) forage mass (1928 kg ha^{-1})

compared with CL (1420 kg ha^{-1}) which could have potentially led to greater animal production per unit land area than on legume treatments (Table 2).

The pastures used in this study had been used to graze cattle on ryegrass in the spring for many years prior to this study. The lack of adequate forage growth compared to what is normally expected in these pastures when they are N fertilized highlighted the challenges producers face for establishing functioning long-term legume-grass grazing systems. The lack of forage growth in the legume treatments was not anticipated to the extent that it occurred. Additionally, data indicate that legumes did not contribute immediately during the first year to the overall N pool in the soil.

Species Composition

Because two different sets of animals were used during spring and summer and because the basal dominant forage differed in these two seasons, data are presented separately for these two seasons. Spring species composition of legumes was affected by treatment (Table 3). The C and CL treatments had the greatest frequency of occurrence of legumes. The L treatment had less ($P < 0.05$) frequency of occurrence of legumes than either C or CL, and Con had 0% occurrence of legumes. White clover matures later in spring than crimson clover explaining the decreased occurrence of clover in treatments containing white clover. Ryegrass, bermudagrass, and all other forages and weeds (OP) were not different ($P > 0.05$) across treatments. This was expected, given the time of data collection at which bermudagrass was still dormant. In addition, this observation indicated that competition from ryegrass did not differ ($P > 0.05$) across treatments.

Similar to spring, summer species composition of legumes was affected by treatments (Table 3). The CL and L treatments did not differ ($P > 0.05$) and had greater ($P < 0.05$) frequency of occurrence of legumes when compared with C and Con treatments which had 0% legume occurrence. Similar to the difference between C and CL treatments in spring, L was numerically greater ($P > 0.05$) than CL. The decline in total clover in CL treatments was likely due to different pasture species sharing a single nutrient pool. With crimson clover being a cool season legume, this species reached maturity earlier in the season and may have been more competitive for nutrients in spring than white clover with its low-growing characteristics. While the combination of crimson and white clover in the CL treatment provided clover throughout spring and summer seasons, the competition between the two species may have decreased the overall occurrence compared to pure stands of either species. Ryegrass, bermudagrass, and other forages and weeds again did not differ ($P > 0.05$) across treatments (Table 1). Treatment C had 95% and 0% legumes in spring and summer, respectively; and L had 34% and 61% legumes in the spring and summer, respectively. However, treatment CL had 78% and 44% legumes in spring and summer, respectively. These percentages show that a combination of crimson and white clover can provide legumes throughout spring and summer. The white clover compensated for the lack of crimson clover in the summer, and the crimson clover supplemented the diminished white clover presence in spring. Coates et al. (1997) stated that legumes mixed in a grass pasture can provide forage production and quality benefits for longer periods of time, given that species close gaps in forage growth over time as in this study.

The species composition results reflected expected changes in pasture species over time. Clovers occurred in respective treatments according to their lifecycle, but it is in general difficult to speculate how inter-species competition may have affected species composition in the CL treatment. For example, white clover in L during summer showed a frequency of occurrence of 61% compared to 44% in CL. However, it would be speculative to infer reasons for the difference other than the ending of the crimson clover lifecycle. Lower legume occurrence in CL could also have been affected by increased weed pressure, but differences between means were not significant to support this assumption.

Forage Chemical Composition

There were no statistically significant differences ($P > 0.05$) among treatments or treatment by month interactions for NDF, ADF, ADL, or N during the spring season (Table 4). During the summer season, there were no treatment by month interactions, and there were no differences among NDF, ADF, or ADL. However, percent N was greatest ($P < 0.05$) for Con compared with C. Nitrogen concentrations in CL and L were intermediate and did not differ ($P > 0.05$) from either Con or C. Animal ADG for the spring season did not differ either ($P > 0.05$), which may be expected for animals consuming forage with fiber and N levels that do not differ. The decrease in N concentration for C in the summer season is most likely due to the absence of crimson clover in the summer season and increasing maturity of bermudagrass. Paddocks containing L showed a greater ($P < 0.05$) frequency of occurrence of legumes which helped the forage in those paddocks maintain N concentrations that did not differ ($P > 0.05$) from the conventionally fertilized pastures. Gettle et al. (1996) stated that legumes used in combination with switchgrass were able to supply N to the switchgrass and improve forage quality. Shaeffer and Evers (2007) also reported that in their study, legumes had the ability to fix N and provide it to other plants through their decomposition in the soil which caused pasture N concentrations not to differ from N-fertilized pastures.

These results indicated that overall, relatively few differences may occur in terms of intra-pasture forage chemical composition under the circumstances of a well-managed forage stand. Although differences are notable between warm and cool season species, both can approach the same N concentrations depending on plant-morphological stage, fertilization, and management. This is another argument for contention of whether legumes should be grown primarily for contributing N or to provide forage high in CP. In the humid southeastern US, it appears that options for providing forage grasses and managing them for high nutritive value are plentiful which may make the development of feasible grass/legume grazing system less of a priority. Conversely, in a more arid environment where grass species survive based on their water-efficient C_4 metabolism that at the same time results in high fiber concentrations, legumes are seen as an option to increase the overall nutritive value of a pasture. As indicated above, to provide substantial amounts of N to a pasture derived from biological fixation, it is necessary to develop strategies for clean-till legume production or within the grass sward that result in higher forage mass production, and thus higher N yields per unit area.

Soil Quality

Although there were seasonal effects on soil parameters detected, no season by treatment interaction was present ($P > 0.05$). Therefore, data were averaged across season, as seasonal differences are expected and the emphasis of this study was on differences between legume treatments.

Treatment Con had greater ($P < 0.05$) levels of dissolved total N, nitrate, ammonium, and inorganic N compared with other treatments (Table 5). This is likely attributed to the greater levels of N fertilizer compared with other treatments. Microbial biomass C and N concentrations were greater ($P < 0.05$) in L than in C (Table 5). Treatments Con and CL were intermediate between L and C and were not different ($P > 0.05$) from each other (Table 5). Dehydrogenase activities were greater ($P < 0.05$) in C than CL. Con and L did not differ ($P > 0.05$) from each other and levels were intermediate the other two treatments (Table 5). This indicated that the soil microbes found in treatment C had a greater ($P < 0.05$) potential for metabolic activity than CL while having the smallest numerical microbial biomass. Although C had the greatest potential metabolic activity, C had the least potential for decomposing the abundant soil polymers chitin and cellulose as indicated by the respective activities of N-acetyl-β-D-glucosaminidase and β-glucosidase. Treatments CL, L, and Con did not differ ($P > 0.05$) from each other and had more N-acetyl-β-D-glucosaminidase enzyme activity than treatment C. Treatment Con was greater ($P < 0.05$) than treatment C while L and CL did not differ ($P > 0.05$) from each other and was between the other two treatments in β-glucosidase enzyme activities. These data suggest L, CL, and Con pastures may contain more microorganisms such as fungi and actinomycetes that decompose polymers which at longer time scales may contribute to humification of soil organic matter (Pierzynski et al., 2000).

Experiment 2

Data analyses indicated a three-way interaction (year by treatment by month) and a two-way interaction (treatment by month) ($P > 0.05$). Therefore, data are presented by year and month. In addition, because each legume species has a distinct growing pattern including different flowering times, data are presented for annuals and perennials differently in a comprehensive manner in Tables 6 – 8 and Tables 11 – 13, respectively.

The data regarding forage mass include only the amount of forage measured when cattle were moved into a pasture at the beginning of each grazing cycle. Since hay rings were not used in order to correct forage removal by cattle, it was determined that reporting ingoing forage would give a representation of the amount of forage mass available to cattle at the beginning of each grazing rotation. Forage production also changed over the course of the season and some species did not survive for the duration of the experiment.

Annual Legume Species Composition and Forage Mass

2008

Crimson clover and hairy vetch treatments did not differ ($P > 0.05$) with respect to frequency of occurrence, and at the beginning of the season, both had a greater ($P < 0.05$) frequency of occurrence than arrowleaf clover (Table 6). Crimson clover and hairy vetch were 90% or greater while arrowleaf clover was between approximately 57% and 62%. Crimson clover and hairy vetch treatments also had greater ($P < 0.05$) forage mass (2768 kg ha^{-1} and 2013 kg ha^{-1}, respectively) early in the season than arrowleaf clover and control treatments (1154 kg ha^{-1} and 727 kg ha^{-1}, respectively) (Table 6 and Figure 1).

Crimson clover is known for having high seedling vigor and tolerates colder temperatures very well. This clover is considered one of the highest-yielding cool season legumes at colder temperatures (Sheaffer and Evers, 2007 and Ball et al., 2007). Hairy vetch is also cold tolerant but does not produce as much forage mass as crimson clover (Sheaffer and Evers, 2007). By July, however, crimson clover and hairy vetch declined numerically in frequency of occurrence (0% and 2% respectively, Table 6) and arrowleaf clover became dominant in frequency of occurrence and forage mass (85% and 5047 kg ha^{-1} respectively, Table 6 and Figure 1).

Although arrowleaf clover matures slowly, it is usually one of the most prolific varieties compared to other annual legumes once it matures (Sheaffer and Evers, 2007). When crimson clover and hairy vetch had a greater ($P < 0.05$) frequency of occurrence early in the season, the occurrence of OP was less ($P < 0.05$) than in the arrowleaf clover and control treatments. When arrowleaf clover became dominant in July, it also had decreased ($P < 0.05$) occurrences of OP compared to control treatments. Crimson clover and hairy vetch legume treatments showed decreased ($P < 0.05$) occurrences of OP than control treatments throughout most of the spring and summer and arrowleaf clover showed decreased ($P < 0.05$) occurrences of OP than control treatments throughout the summer (Table 6).

2009

Arrowleaf clover frequency of occurrence did not differ from crimson clover and hairy vetch treatments in March (Table 7). This abundance of arrowleaf clover in March may have been related to its reseeding ability. This species has been reported to produce approximately 90% hard seed (Sheaffer and Evers, 2007). Hard seeds are more likely to germinate in the fall than summer, thus, giving seedlings a better chance of survival until the next spring/early summer to complete their vegetative and reproductive growth cycle. However, arrowleaf reseeding potential may have been reduced by the more than doubled frequency of occurrence of bermudagrass in November 2009 compared to 2008.

Crimson clover treatments had a numerically decreased frequency of occurrence of legumes in 2009 compared to 2008 (Table 7). This may have been an indicator of diminished reseeding ability. Arrowleaf clover appeared to persist longer through the summer than the other two legume treatments since crimson clover and hairy vetch are better suited to cooler temperatures. The crimson clover treatment also showed a greater ($P < 0.05$) frequency of occurrence of OP than the arrowleaf clover treatments for all sample dates except during the last assessment in November when all legumes grew at a reduced rate (Table 7). The decrease in November legume population in 2009 may have been related to increased frequency of occurrence of bermudagrass. This increase in bermudagrass might have created competition for nutrients between the legumes and bermudagrass which negatively impacted legume frequency of occurrence.

Bermudagrass frequency of occurrence did not differ ($P > 0.05$) among all treatments throughout 2009 (Table 7). In May, frequency of occurrence of bermudagrass ranged from 25.2% to 52.6% due to variation in bermudagrass, but in later months, that frequency of occurrence rose to nearly 100% across all treatments. There were no differences ($P > 0.05$) in forage mass across treatments for any sample date in 2009 despite the presence or absence of legumes (Figure 2).

2010

Crimson clover was not sampled in 2010. Due to a lack of reseeding it was not present in its respective treatments. Arrowleaf clover also declined numerically in frequency of occurrence early in the year in comparison to 2009. In April and May of 2010, arrowleaf clover frequency of occurrence was 45.5% and 76.2%, respectively, (Table 8) compared to 93.2% and 91.3% in March and May of 2009 (Table 7). Hairy vetch, however, maintained a stand and had a greater ($P < 0.05$) frequency of occurrence compared to arrowleaf clover until the hairy vetch population decreased at the end of its life cycle in the summer months (Table 8). The hairy vetch treatment also showed a decreased ($P < 0.05$) frequency of occurrence of OP than the control and arrowleaf clover plots in April and May (Table 8). This decrease in other plants may have been a result of the thick growth of hairy vetch early in the growing season. Hairy vetch had greater forage mass ($P < 0.05$) than the control treatment in April but not in May where the control was greater ($P < 0.05$) in forage mass (Figure 3). The increased frequency of occurrence of OP in the control pastures, which could have consisted of dense weeds, likely played a role in the increased forage mass of control pastures in May.

Annual Legume Chemical Composition

Plant chemical composition data were available only for years 2008 and 2009. All three legume treatments had less ($P < 0.05$) NDF and greater ($P < 0.05$) N concentrations than the control in April 2008 when they had a high occurrence of legumes and were at a highly digestible stage of maturity (Table 9). As a result of plant maturity, all fiber levels increased and N levels decreased numerically ($P > 0.05$) from April 2008 to July 2008 (Table 9). Crimson clover and hairy vetch declined in frequency of occurrence in July, and they were not as different to the control in terms of species composition and therefore presumably were not as different in their NDF levels as well. Arrowleaf clover still had a high frequency of occurrence in July causing the treatment to have less ($P < 0.05$) NDF than the other treatments, but arrowleaf clover showed numerically greater ($P > 0.05$) ADF and ADL concentrations and decreased N ($P < 0.05$) compared to April (Table 9). The control treatment had decreased ADF and ADL ($P < 0.05$) compared to the other three treatments in July (Table 9). Mature legume plant tissue in crimson clover, hairy vetch, and arrowleaf clover samples from July could have increased the ADF and ADL concentrations for those treatments. The maturity stage of arrowleaf clover in July was most likely responsible for its increased ADF and ADL levels compared to the control that perhaps contained plant species that were not as advanced in their maturity as arrowleaf clover.

In 2009, NDF concentration was once again decreased ($P < 0.05$) and N concentrations were greater ($P < 0.05$) in legume treatments until June when crimson clover and hairy vetch legume frequencies declined (Table 10). Crimson clover, hairy vetch, and arrowleaf clover also had greater ($P < 0.05$) ADL concentrations in May than the control. Legumes were in a late stage of growth at this time which may explain the increased lignification. Arrowleaf clover had decreased NDF concentrations in June ($P < 0.05$) compared with the other treatments. However, arrowleaf clover was apparently not mature enough in June to have greater ADF and ADL concentrations than the control as it did in July of 2008 (Table 9). Arrowleaf clover had decreased ($P < 0.05$) concentrations of NDF in May and June and greater ($P < 0.05$) concentrations of N in May compared to crimson clover in 2009 (Table 10). This agrees with Ball et al. (2007), who stated that arrowleaf clover can consistently produce greater quality forage compared to crimson clover.

Perennial Legume Species Composition and Forage Mass Production

2008

Legume frequency of occurrence did not differ ($P > 0.05$) between all legume treatments until August when alfalfa frequency declined compared to red clover (Table 11). Frequency of occurrence for bermudagrass was often numerically less ($P > 0.05$) in legume treatments compared to the control. Red clover and white clover seemed to have been better at suppressing the occurrence of OP in August and September than the alfalfa treatment which usually did not differ ($P > 0.05$) from the control in that respect (Table 11).

There were no differences ($P > 0.05$) in forage mass in June (Figure 4), but in July, alfalfa and red clover produced more ($P < 0.05$) forage mass (2162 kg ha^{-1} and 2311 kg ha^{-1}, respectively, Figure 4) than white clover and control (1316 kg ha^{-1} and 1241 kg ha^{-1}, respectively, Figure 4). Alfalfa and red clover produced more DM than white clover due to the fact that alfalfa and red clover have larger stems, and the control did not have the added benefit of legumes to add to its forage mass. Red clover may provide more grazing biomass than white clover in the summer (Ball et al., 2007). In August, treatments did not differ ($P > 0.05$) in forage mass. In September, the control had the greatest ($P < 0.05$) forage mass (4181 kg ha^{-1}), and white clover had decreased ($P < 0.05$) forage mass compared with all other treatments.

2009

Alfalfa did not persist well as evidenced by its decreased ($P < 0.05$) frequency of occurrence on each sampling date in 2009 (Table 12) compared with the other legume treatments. This was perhaps due to stress caused by weevils. Weevil damage was observed on some alfalfa plants in early 2008 that affected young plants that died shortly after. White clover had a numerically greater ($P > 0.05$) frequency of legume occurrence in 2009, and red clover also maintained its frequency (Table 12). The frequency of occurrence of bermudagrass was again at least numerically less ($P > 0.05$) in the white clover and red clover treatments on most sample dates (Table 12). However, the alfalfa treatment frequencies of occurrence of bermudagrass and OP did not differ ($P > 0.05$) compared to control treatments, probably due to the lack of competition from alfalfa plants that had declined in frequency. The red clover and white clover treatments consistently had decreased ($P < 0.05$) frequency of OP compared to the control (Table 12). Red clover had greater ($P < 0.05$) forage production than white clover in May and June

(Figure 5) which is expected, because red clover produces a taller plant with larger stems than white clover. In August and September, red clover and white clover forage mass dropped below the control and alfalfa treatments which did not differ ($P > 0.05$) from each other (Figure 5). This was most likely because alfalfa and control treatments had greater ($P < 0.05$) frequencies of occurrence of bermudagrass than the other two treatments in late summer when legumes had less of an impact on forage mass.

2010

Like crimson clover in the annual treatments, alfalfa plots no longer contained alfalfa in 2010. Therefore, data were collected only for red clover, white clover, and control treatments. Legume persistence seemed to decline for both red clover and white clover in 2010 (Table 13). The frequency of occurrence in both treatments was high (at least 88.0%) until June. In July, white clover and red clover dropped in occurrence to 59.3% and 48.4%, respectively (Table 13), whereas their occurrence had remained high in 2009 throughout the summer. The bermudagrass had to compete less with legumes than it had in past years allowing it to be more abundant. However, the frequency of occurrence of OP was lower in the two legume treatments compared to the control in May and June (Table 13). In April, the red clover treatment had the greatest ($P < 0.05$) forage mass production (Figure 6). This was probably a result of the lack of bermudagrass at the time and that red clover generally grows taller and has much larger thicker stems than white clover. Forage mass was not different ($P > 0.05$) among treatments in May and June (Figure 6). While control pastures lacked clovers in May and June, there was an abundance of OP which most likely contributed to the lack of differences in forage mass among treatments. In July and August, forage mass from control was greater ($P < 0.05$) than that from white clover.

The presence of white clover earlier in the growing season may have somewhat depleted the nutrients available to bermudagrass in those experimental pastures which could have decreased overall forage production. Red clover might have also had decreased forage mass if not for the decaying stems of the red clover plants that were still present at these samples periods. In September, forage biomass did not differ ($P > 0.05$) between treatments.

Perennial Legume Chemical Composition

Plant chemical composition data were available for years 2008 and 2009. Red clover treatments often had the greatest frequency of occurrence of legumes and lowest frequency of occurrence of other plants. Red clover consistently had less ($P < 0.05$) NDF than the control and greater ($P < 0.05$) N concentrations compared to control treatments in July through September, 2008 (Table 14). This was not the case for the alfalfa and white clover treatments which did not differ ($P > 0.05$) from the control in NDF and N concentrations on several sample dates in 2008 (Table 14). The increase in frequency of occurrence of white clover from 2008 to 2009 was also evident by analyzing forage chemical composition data. White clover consistently had decreased NDF ($P < 0.05$) and increased N concentrations ($P < 0.05$) compared to the control in 2009 (Table 15). Red clover also consistently had decreased NDF ($P < 0.05$) compared to the control. Red clover had greater ($P < 0.05$) N concentrations than the control in May and June, but N concentration between red clover and control pastures did not differ in August ($P > 0.05$). If red clover was present in pastures in August, it was most likely not actively growing and was in a state of decay. This would also help explain the increased ADL concentration ($P < 0.05$) in red clover pastures compared to all other treatments in August.

Soil Quality Parameters for Annual Legumes

No treatment by season interaction was observed ($P > 0.05$), but treatments had an effect on some of the soil parameters tested (Table 16). Crimson clover treatments had greater organic matter ($P < 0.05$), microbial biomass C concentrations ($P < 0.05$), dehydrogenase activities ($P < 0.05$), and C and N cycling enzyme activities ($P < 0.05$) than hairy vetch. Microbes in crimson clover pastures may have been able to better utilize the decaying plant matter of crimson clover than that of the other annual species which might explain the increased organic matter and biomass accumulation. These data suggest that crimson clover treatments could have more rapid decomposition of abundant non-humic carbon and N containing compounds than hairy vetch treatments. These compounds are in a pool of organic matter that is decomposed more quickly than the passive pool of soil humus (Brady and Weil, 2008). Hairy vetch also had greater ($P < 0.05$) plant concentrations of N while having the least soil microbial enzymatic activity. This could mean that hairy vetch was better outcompeting microorganisms for soil nutrients. Crimson clover treatments also had a greater level of dissolved organic carbon ($P < 0.05$), dissolved organic N ($P < 0.05$), and ammonium ($P < 0.05$) than the arrowleaf clover treatment, but of the enzymes, only dehydrogenase activities were significantly greater ($P < 0.05$). A greater level of dehydrogenase activities indicates greater levels of aerobic metabolic activity in crimson clover treatments ($P < 0.05$) than the other two legume treatments. While the hydrolytic enzyme activities involved in cellulose and chitin decomposition were not greater ($P > 0.05$), the dehydrogenase and dissolved organic C and N concentrations suggest that nutrient cycling from decomposition and N mineralization may be greater in treatments with crimson clover than arrowleaf clover.

Soil Quality Parameters for Perennial Legumes

In the case of the perennials, the alfalfa treatments had greater levels ($P < 0.05$) of various parameters than the control (Table 17). However, the actual presence of alfalfa in the pastures was low and thus the differences in the data may not have been representative of the effect that the presence of alfalfa had and could possibly have instead been the product of an outside source or a previous treatment to the experimental pastures before the initiation of this study. However, the alfalfa could have established a strong root system which began to decay with the declining frequency of occurrence of the alfalfa. This decaying root system could have provided the catalyst for increased ($P < 0.05$) OM in alfalfa pastures. The white clover treatment had greater ($P < 0.05$) dissolved organic N than the control treatments. This increased soil organic N may have resulted from the increased plant N concentration in white clover pastures. All three legume treatments had greater glucosidase enzyme activities ($P < 0.05$) than the control (Table 17), suggesting increased decomposition of plant matter. However, the lack of differences in most parameters indicates there was not a strong effect of immediate biological and biochemical improvement.

Summary of Experiment 1 and 2

In order to accurately determine our experimental legumes' impact on the pasture environment, there are other limiting conditions that need to be addressed. To properly measure potential legume production, it would have been beneficial to have had some form of weed control. This likely would have limited competition against legumes from more aggressive plant species allowing legumes more access to pasture nutrients for maximized growth. It was also difficult to determine the extent to which legumes actually contributed to the pasture chemical composition and soil nutrient pool since there were plant species other than legumes present in all pastures. With a weed control in place likely limiting the species diversity of pastures, it would have been more feasible to draw conclusions about which plants made the largest contribution to pasture chemical composition and the soil nutrient pool.

The annual legume treatments had less ($P < 0.05$) frequency of occurrence of OP, at almost every sample date over the three years of the study. This provided evidence that legumes in pasture were not able to completely eliminate weeds, but legumes were able to provide some weed control when compared to pastures that did not contain legumes and were not sprayed with conventional weed-controlling chemicals. This is corroborated by Ross et al. (2001) who stated that clovers had weed-controlling benefits. However, effective weed control via an additional pasture species depends on canopy control, morphology, and grazing of paddocks. In this study, diminishing legume abundance appeared to result in a visibly increased weed occurrence in many experimental units. In general, weed control is one of the most important challenges that needs to be addressed in grazing systems with a legume component. However, chemical weed control is difficult due to the lack of available selective herbicides.

Our measurement of frequency of occurrence only provided an approximation of the presence of legumes. A more accurate but more laborious method of determining pasture legume content is the total separation of species by hand which was not feasible for this study given the large number of samples.

In experiment 1, control pastures either did not differ ($P > 0.05$) or had greater ($P < 0.05$) plant N concentrations than legume pastures. If additional N fertilizer was not used in control pastures, there may have been a difference in plant N concentration. In experiment 2, there was greater N concentration ($P < 0.05$) in plant chemical composition for pastures containing legumes compared to control pastures. No additional N fertilizer was used in experiment 2 which suggested that the increased plant N concentration was a result of legume presence in respective pastures. As a result, both experiments indicated that legumes can contribute to overall pasture quality.

Using one species of legume per pasture appeared to have an effect on the population of other plants in the pastures. For future research, it would be beneficial to conduct an experiment similar to experiment two measuring persistence while combining different species in the same plots similar to the CL plots from experiment one. Using species that have different periods of productivity within the same plots could possibly create competition for undesirable species. Performing experiments similar to experiment one and two for longer periods of time might also yield different results. It would certainly be of interest to observe legume persistence over longer periods and whether the contribution of legumes to forage chemical composition and soil parameters persisted after the legume occurrences declined or legumes disappeared entirely.

Conclusion

Various studies have been conducted in the past to determine the persistence of forage legumes and their effect on animal performance in grazing systems. Legumes have shown to be site-specific and adaption of a single or more species to a wide geographic region is difficult.

Based on the results of experiment 1, the use of white clover and crimson clover in pastures may result in ADG that does not differ ($P > 0.05$) from a grass-only pasture that is fertilized using synthetic N. It is also possible that total BW gains may not be different ($P > 0.05$) between animals that graze grass-legume mixed swards and animals that graze grass pastures fertilized with synthetic nitrogen under equal grazing days. However, since legumes are site-specific, results from this study cannot easily be extrapolated to other regions even in close approximation to the research area.

Reduced numbers of grazing days available for legume treatments appear to be a major drawback in establishing a legume-based grazing system. This has implications for producer acceptance, since a delay in grazing, at least during the initial phase of establishing such a system, may result in financial losses. In addition, it may take several years before soil N pools have substantially increased to offset N fertilizer costs. In order to transport significant amounts of N to other plants, legumes must either complete their life cycle and be recycled into the soil or be grazed by livestock and reapplied to pastures as fecal or urinary deposits. While legumes may improve the nutritive value of pastures and provide animal performance that does not differ from well fertilized grasses, legumes did not appear to be able to entirely replace the need for N fertilizer in experiment 1.

Experiment 2 suggested that in the specific geographic location near Batesville, AR, it is necessary to establish a comprehensive weed control program to keep legumes part of warm-season grass pastures. In this experiment, white clover, red clover, and hairy vetch persisted longer than the other legume species, including alfalfa, arrowleaf clover, and crimson clover. The data suggested that the legume species that did not persist well may have provided an extra source of nutrients for the soil from their decaying root systems. In general, the longevity of legumes is reduced in the warm and humid environment present in the southern U.S. More importantly, successful establishment and utilization of legumes will depend on improved grazing management practices.

References

Bagwell, C. E., Y. M. Piceno, A. Ashburne-Lucas, and C. R. Lovell. 1998. Physiological Diversity of the Rhizosphere Diazotroph Assemblages of Selected Salt Marsh Grasses. Applied and Environmental Microbiology. 64:4276-4282.

Ball, D. M., C. S. Hoveland, and G. D. Lacefield. 2007. Southern Forages 4th ed. International Plant Nutrition Institute, Norcross, Georgia.

Bergersen, F. J. 1982. Root nodules of legumes: Structure and functions. Letchworth, Herts. SG6 3BE, England: Research Studies Press.

Bhatti, S. A., J. G. P. Bowman, J. L. Firkins, A. V. Grove, and C. W. Hunt. 2008. Effect of intake level and alfalfa substitution for grass hay on ruminal kinetics of fiber digestion and particle passage in beef cattle. Journal of Animal Science. 86(1): 134-145.

Blaser, R. E., T. Taylor, W. Griffeth, and W. Skrdla. 1956. Seedling competition in establishing forage plants. Agronomy Journal. 48:1–6.

Brady, N.C., and R. Weil. 2001. The nature and properties of soil. Prentice Hall, 13th Edition. Upper Saddle River, NJ.

Broderick, G. A. 1995. Desirable characteristics of forage legumes for improving protein utilization in ruminants. Journal of Animal Science. 73:2760-2773.

Brown, L. R. 1999. Feeding nine billion. Page 115 in State of the World 1999. A World Watch Institute Report on Progress Toward a Sustainable Society. Brown L. R., C. Flavin and H. French, eds. W. Norton & Company, NY.

Burns, J. C., and J. E. Standaert. 1985. Productivity and economics of legume-based vs. nitrogen-fertilized grass-based pasture in the United States. In: R. F. Barnes, P. R. Ball, R. W. Brougham, G. C. Marten, and D. J. Minson (ed.) Forage Legumes for Energy-Efficient Animal Production. Proc. Trilateral Workshop, Palmerston.

Cabrera, M.L., and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal. 57:1007-1012.

Casida, L.E., Jr., D.A. Klein, and T. Santora. 1964. Soil Dehydrogenase Activity. Soil Science. 98:371-376.

Coates, D. R., C. P. Miller, R. E. Hendricksen, and R. J. Jones. 1997. Stability and productivity of stylosanthes pastures in Australia. II. animal production from stylosanthes pastures. Tropical Grassland. 31(5): 494-502.

Crews, T. E., and M. B. Peoples. 2004. Legume versus fertilizer sources of nitrogen: Ecological tradeoffs and human needs. Agriculture, Ecosystems & Environment. 102(3): 279-297.

Drinkwater, L. E., and P. Wagoner. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature. 396(6708): 262-265.

Dueck,T. A., F. G. Dorèl, R. Ter Horst and L. J. M. Van der Eerden. 1990. Effects of ammonia, ammonium sulphate and sulphur dioxide on the frost sensitivity of Scots pine (*Pinus sylvestris* L.). Water, Air, & Soil Pollution. 54: 35-49.

Eekeren, N., D. Liere, F. Vries, M. Rutgers, R. Goede, and L. Brussaard. 2009. A mixture of grass and clover combines the positive effects of both plant species on selected soil biota. Applied Soil Ecology. 42:254-263.

Evers, G. W. (Unpublished). What does it take to grow cool season legumes. Texas A&M University Agricultural Research and Extension Center at Overton.

Fangmeier, A., A. Hadwiger-Fangmeier, L. Van der Eerden and H. J. Jäger. 1994. Effects of Atmospheric Ammonia on Vegetation - A Review. Environmental Pollution. 86: 43-82.

Foster, J. L., A. T. Adesogan, J. N. Carter, A. R. Blount, R. O. Myer and S. C. Phatak. 2009. Intake, digestibility, and nitrogen retention by sheep supplemented with warm-season legume hays or soybean meal. Journal of Animal Science. 87:2891-2898.

Galloway, J. N., E. B. Cowling, S. P. Seitzinger, and R. H. Socolow. 2002. Reactive Nitrogen: Too Much of a Good Thing? Ambio. 31:60-63.

Gettle, R. M., D. R. Buxton, K. J. Moore, J. R. George, and K. M. Blanchet. 1996. Frost-seeding legumes into established switchgrass: Establishment, density, persistence, and sward composition. Agronomy Journal. 88(1): 98-103.

Greenquist M. A., T. J. Klopfenstein, W. H. Schacht, G. E. Erickson, K. J. Vander Pol, M. K. Luebbe, K. R. Brink, A. K. Schwarz, and L. B. Baleseng. 2009. Effects of nitrogen fertilization and dried distillers grains supplementation: Forage use and performance of yearling steers. Journal of Animal Science. 87:3639-3646.

Harmoney, K. R., C. L. Burras, J. R. George, K. J. Moore, and E. C. Brummer. 2001. Spatial legume composition and diversity across seeded landscapes. Agronomy Journal. 93(5): 992-1000.

Hill, J. O., M. J. Robertson, B. C. Pengelly, R. L. Clem, D. B. Coates, and A. M. Whitbread. 2009. Seasonal changes in pasture quality and diet selection and their relationship with liveweight gain of steers grazing tropical grass and grass-legume pastures in northern australia [electronic resource]. Animal Production Science. 49(11): 983-993.

Hoveland, C. S., J. H. Bouton, and M. A. McCann. 1997. Influence of endophyte, alfalfa, and grazing pressure on steer performance and plant persistence of jesup tall fescue. Journal of Production Agriculture. 10(4): 546-550.

Huneycutt, H. J., C. P. West, and J. M. Phillips. 1988. Responses of bermudagrass, tall fescue and tall fescue-clover to broiler litter and commercial fertilizer. Bulletin, Arkansas Agricultural Experiment Station, Arkansas University. (913): 20-20.

Jones, D.L., and V.B. Willett. 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology and Biochemistry. 38:991-999.

Karam, A. 1993. Chemical Properties of Organic Soils, p. 459-471, *In* M. R. Carter, ed. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Ottawa, Ontario Canada.

Kennedy, I. R., 1992. Acid Soil and Acid Rain, second ed. Wiley, New York.

Kjaergaard, Thorkild. 2003. A Plant that Changed the World: The rise and fall of clover 1000-2000. Landscape Research, 28:1, 41-49.

Lauriault, L. M., R. E. Kirksey, G. B. Donart, J. E. Sawyer, and D. M. Van Leeuwen. 2005. Pasture and Stocker Cattle Performance on Furrow-Irrigated Alfalfa and Tall Wheatgrass Pastures. Crop Science. 45: 305–315.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. Animal Feed Science Technology. 57:347-358.

Lindemann, W. C. and C. R. Glover. 2003. Nitrogen Fixation by Legumes. New Mexico State University Extension Guide, A-130.

Lomas, L. W., J. L. Moyer, G. L. Kilgore, and G. A. Milliken. 2004. Effects of Grazing on Legume Persistence, Forage Production, and Steer Performance in Endophyte-Infected Tall Fescue Pastures. The Professional Animal Scientist, 20: 46-52.

Mosier, A. R., M. A. Bleken, P. Chaiwanakupt, E. C. Ellis, J. R. Freney, R. B. Howarth, P. A. Matson, K. Minami, R. Naylor, K. N. Weeks, and Z. L. Zhu. 2001. Policy implications of human accelerated nitrogen cycling. Biogeochemistry 52:281-320

Mouriño, F., K. A. Albrecht, D. M. Schaefer, and P. Berzaghi. 2003. Steer performance on kura clover-grass and red clover-grass mixed pastures. Agronomy Journal. 95(3): 652-659.

Mulvaney, R.L. 1996. Nitrogen - Inorganic Forms, p. 1123-1184, In D. L. Sparks, ed. Methods of Soil Analysis: Chemical Methods. Part 3. Soil Science Society of America, Inc., Madison, WI.

Nelson, C. J. and L. E. Moser. 1994. Plant Factors Affecting Forage Quality. Page 115 in Forage Quality, Evaluation, and Utilization. G. C. Fahey, Jr. ed. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WI.

North, NZ. pp 56–71.Coates, D. R., C. P. Miller, R. E. Hendricksen, and R. J. Jones. 1997. Stability and productivity of stylosanthes pastures in Australia. II. animal production from stylosanthes pastures. Tropical Grasslands. 31(5): 494-502.

NRC. 1993. Soil and Water Quality, An Agenda for Agriculture, Committee on Long-Range Soil and Water Conservation, Board on Agriculture, National Research Council, National Academy Press, Washington, DC.

Owens, L. B., R. W. Van Keuren, and W. M. Edwards. 1994. Groundwater nitrate levels under fertilized grass and grass-legume pastures. Journal of Environmental Quality. 23(4): 752-758.

Parham, J. A. and S.P. Deng. 2000. Detection, Quantification and Characterization of β-glucosaminidase Activity in Soil. Soil Biology & Biochemistry. 32: 1183-1190.

Peoples, M. B., Freney, J. R., Mosier, A. R., 1995. Minimizing gaseous losses of nitrogen. In: Bacon, P. E. (Ed.), Nitrogen Fertilization in the Environment. Marcel Dekker, New York, pp. 565-602.

Pierzynski, G. M., J. T. Sims, and G. F. Vance. 2000. Soils and Environmental Quality 2nd ed. CRC Press LLC. Boca Raton, Florida.

Robertson, M. J. _. 2000. Using Models to Assess the Value of Traits of Food Legumes from a Cropping Systems Perspective. Pages 265-278 in Linking Research and Marketing Opportunities for Pulses in the Twenty-First Century: Proceedings of the Third International Food Legumes Research Conference. Knight, R., ed. Current Plant Science and Biotechnology in Agriculture, vol. 34; Dordrecht; Boston and London: Kluwer Academic.

Robinson, G. S. and W. A. Jacques. 1958. Root development in some common New Zealand pasture plants: effect of pure sowings of some grasses and clovers on the structure of a Tokomaru silt loam. New Zealand Journal of Agricultural Research. 1:199-216.

Rochon, J. J., C. J. Doyle, J. M. Greef, A. Hopkins, G. Molle, M. Sitzia, D. Scholefield, and C. J. Smith. 2004. Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. Grass and Forage Science. 59: 197–214.

Ross, Shirley, J. R. King, R. C. Izaurraldeb, and J. T. O'Donovanc. 2001. Weed Suppression by Seven Clover Species. Agronomy Journal. 93: 820-827.

Schindler, D. W., 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnology and Oceanography. 23: 478-486.

Sheaffer, C. C. and G. W. Evers. 2007. Cool-season Legumes for Humid Areas. Page 179 in Forages: The Science of Grassland Agriculture Vol. II. 6th rev. ed. R. F. Barnes, C. J. Nelson, K. J. Moore, and M. Collins eds. Blackwell Publishing, Ames, IA.

Silva, George. 2011. Fertilizer Prices on the Rise. Accessed April 21, 2011. http://www.ipmnews.msu.edu/fieldcrop/fieldcrop/tabid/56/articleType/ArticleView/articleId/3269/Fertilizer-prices-on-the-rise.aspx.

Smil, V. 1990. Nitrogen and phosphorus, in: Turner, B. L. II, Clark, W. C., Kates, R. W., Richards, J. F., Mathews, J. T., Meyer, W. B. & Jordan, M. (Eds) The Earth As Transformed by Human Action. Global and regional changes in the biosphere over the past 300 years, pp. 423-436. Cambridge, Cambridge University Press.

Smil, V. 2001. Enriching the Earth. MIT Press, Cambridge, MA.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Available online at http://soils.usda.gov/technical/classification/osd/index.html. Accessed [3/6/2011].

Sprent, J. I. 2007. Evolving ideas of legume evolution and diversity: A taxonomic perspective on the occurrence of nodulation. New Phytologist. 174(1): 11-25.

Tabatabai, M.A. 1994. Soil enzymes, p. 775-833, In R. W. Weaver, ed. Methods of Soil Analysis: Microbial and Biochemical Properties. Part 2. Soil Science Society of America, Inc., Madison, WI.

Taylor, R. W., and D. W. Allinson. 1983. Legume establishment in grass sods using minimum-tillage seeding techniques without herbicide application: Forage yield and quality. Agronomy Journal. 75:167–172.

United States Climate Data. Climate-Batesville-Arkansas [Data files]. Available from http://www.usclimatedata.com/climate.php?location=USAR0033. Accessed [3/6/2011].

United States Department of Agriculture Economic Research Service. U.S. fertilizer use and price [Data files]. Accessed April 22, 2011.http://www.ers.usda.gov/Data/FertilizerUse/

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74:3583-3597.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry. 19:703-707.

Vasiljevic´, S., D. Milic´, and A. Mikic´. 2009. Chemical attributes and quality improvement of forage legumes. Biotechnology in Animal Husbandry. 25(5): 493-504.

Vitousek, P. M., J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Causes and Consequences. Issues in Ecology. 1:1-15.

Vogel, K. P., J. F. Pedersen, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Science. 39:276-279.

Vogel, K.P., and R Masters. 2001. Frequency grid—a simple tool for measuring grassland establishment. Journal of Range Management. 54:653-655.

Wedin, D. A. and M. P. Russelle. 2007. Nutrient Cycling in Forage Production Systems. Page 137 in Forages: The Science of Grassland Agriculture Vol. II. 6th rev. ed. R. F. Barnes, C. J. Nelson, K. J. Moore, and M. Collins eds. Blackwell Publishing, Ames, IA.

Whyte, R. O., G. Nilsson-Leissner, and H. C.Trumble. 1953. Legumes in agriculture. Rome, Italy: Food and Agriculture Organization of the United Nations.

Figure 1. Experiment 1: Timeline of grazing, fertilization, and soil sampling events.

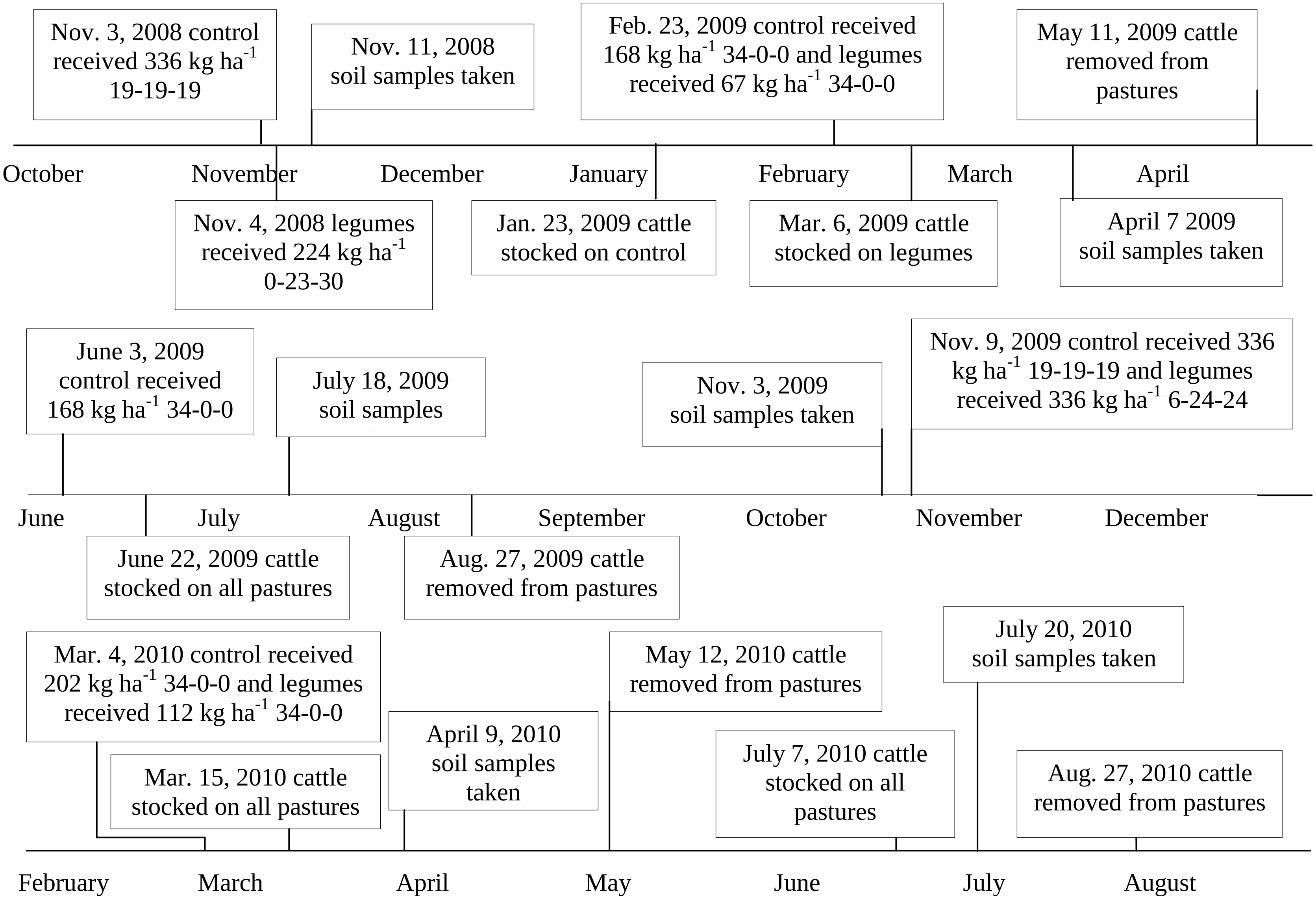


Table 1. Experiment 1: Animal performance during spring and summer as affected by legumes over-seeded the previous fall.

Item	Treatment[2]				SEM[3]
	C	CL	Con.	L	
Spring[4]					
Initial Wt. (kg)	266	267	256	265	24.2
End Wt., (kg)	349	348	354	345	19.8
Total Gain, (kg)	82.6^{b}	80.9^{b}	98.4^{a}	80.5^{b}	6.94
Grazing Days	66^{b}	66^{b}	79^{a}	66^{b}	5.8
ADG[5] (kg/day)	1.3	1.2	1.3	1.2	0.15
Summer[6]					
Initial Wt. (kg)	271	274	274	271	21.3
End Wt., (kg)	301	296	309	297	21.5
Total Gain, (kg)	29.9	22.3	35.8	26.2	6.41
Grazing Days	68	68	68	68	0.0
ADG (kg/day)	0.4	0.3	0.5	0.4	0.10

[1] Two separate sets of animals were used for grazing. Therefore, spring and summer grazing is displayed separately.

[2] C, crimson clover; CL, crimson clover and white clover mixture; Con., Control; L, white clover.

[3] Standard error of the mean.

[4] The spring season contains years 2009, 2010, and 2011.

[5] Average daily gain.

[6] The summer season contains years 2009 and 2010.

[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 2. Experiment 1: Pasture dry matter (DM) forage production and removal during spring and summer as affected by legumes over-seeded the previous fall[1].

Item[2]	Treatment[3]				
	C	CL	Con.	L	SEM[4]
Spring[5]					
Forage Mass[6] (kg/ha)	3254	3063	3540	3448	195.4
Forage Removal (kg/hd/day)	30.6	33.5	35.3	43.3	4.38
Total Forage Growth (kg/ha)	6060	5822	5797	6338	283.3
Summer[7]					
Forage Mass[6] (kg/ha)	1778^{ab}	1420^{b}	1928^{a}	1561^{ab}	97.3
Forage Removal (kg/hd/day)	31.1	22.0	30.9	22.7	4.04
Total Forage Growth (kg/ha)	2674	2049	2788	2253	272.4

[1] Two separate sets of animals were used for grazing. Therefore, spring and summer grazing is displayed separately.

[2] All items reported are on a DM basis.

[3] C, crimson clover; CL, crimson clover and white clover mixture; Con., Control; L, white clover.

[4] Standard error of the mean.

[5] The spring season contains years 2009, 2010, and 2011.

[6] Forage available at the beginning of each grazing cycle.

[7] The summer season contains years 2009 and 2010.

[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 3. Experiment 1: Composition of ryegrass, bermudagrass, legumes, and other pasture plants during spring and summer as affected by crimson clover and white clover over-seeded the previous fall[1].

	Treatment[2]				
Species	C	CL	Con.	L	SEM[3]
	Frequency of occurrence (%)				
Spring[4]					
Ryegrass	100	100	100	100	0.0
Bermuda	1	1	4	6	2.8
Legume	95^a	78^a	0^c	34^b	7.4
OP[5]	30	50	48	48	15.3
Summer[6]					
Ryegrass	2	0	0	0	1.3
Bermuda	55	75	54	85	16.2
Legume	0^b	44^a	0^b	61^a	7.3
OP	78	68	73	52	11.1

[1] Two separate sets of animals were used for grazing. Therefore, spring and summer grazing is displayed separately.

[2] C, crimson clover; CL, crimson clover and white clover mixture; Con., Control; L, white clover.

[3] Standard error of the mean.

[4] The spring season contains years 2009, 2010, and 2011.

[5] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category "other".

[6] The summer season contains years 2009 and 2010.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 4. Experiment 1: Forage chemical composition during spring and summer as affected by legumes over-seeded the previous fall[1].

Item[2]	Treatment[3] C	CL	Con	L	SEM[4]
Spring[5]					
NDF[6] (%)	48.5	46.6	53.1	47.7	2.52
ADF[7] (%)	25.3	24.4	26.7	25.2	1.85
ADL[8] (%)	1.5	2.0	1.4	1.4	0.39
N (%)	2.7	2.9	3.1	2.8	0.41
Summer[9]					
NDF (%)	66.7	65.3	64.5	67.1	1.24
ADF (%)	33.8	31.7	32.4	32.6	0.55
ADL (%)	3.0	3.1	3.1	3.3	0.48
N (%)	2.0^{b}	2.2^{ab}	2.5^{a}	2.1^{ab}	0.24

[1] Two separate sets of animals were used for grazing. Therefore, spring and summer grazing is displayed separately.

[2] All items reported are on a DM basis.

[3] C, crimson clover; CL, crimson clover and white clover mixture; Con., Control; L, white clover.

[4] Standard error of the mean.

[5] The spring season contains years 2009, 2010, and 2011.

[6] Neutral detergent fiber.

[7] Acid detergent fiber.

[8] Acid detergent lignin.

[9] The summer season contains years 2009 and 2010.

[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 5. Experiment 1: Soil quality parameters (dry matter based) across fall, spring, and summer as affected by over-seeded legumes. There was no season by treatment interaction detected ($P > 0.05$). Although there were seasonal effects on some parameters observed, the emphasis was placed on the legume treatment comparison as this was the main research objective.

	Treatment[1]				
Item	C	CL	Con	L	SEM[2]
Gravimetric Soil Moisture, (g g^{-1})	0.20	0.18	0.20	0.19	0.019
Organic Matter (%)	5.49	6.10	6.06	5.84	0.214
DOC[3] (µgC g^{-1})	63	73	68	68	5.4
DTN[4] (µgN g^{-1})	29.3^{b}	27.9^{b}	57.9^{a}	30.3^{b}	4.68
NO_3 (µgN g^{-1})	13.9^{b}	14.3^{b}	27.7^{a}	14.1^{b}	4.01
NH_4 (µgN g^{-1})	3.8^{b}	3.9^{b}	26.6^{a}	4.5^{b}	5.42
Inorganic N (µgN g^{-1})	18.3^{b}	18.7^{b}	48.3^{a}	19.8^{b}	5.40
DON[5](µgN g^{-1})	11.3	9.2	11.4	10.5	1.37
Microbial Biomass C[6] (µgC g^{-1})	189^{b}	205^{ab}	217^{ab}	234^{a}	11.0
Microbial Biomass N[7] (µgN g^{-1})	24.2^{b}	26.6^{ab}	26.8^{ab}	30.8^{a}	2.09
Microbial Biomass C/N[8]	8.5	8.3	9.1	7.9	0.74
DHase[9] (µgTPF g^{-1} 24 hr^{-1})	151^{a}	84^{b}	105^{ab}	138^{ab}	18.8
NAGase[10] (µg p-nitrophenol g^{-1} hr^{-1})	52^{b}	70^{a}	73^{a}	73^{a}	5.1
PNGase[11] (µg p-nitrophenol g^{-1} hr^{-1})	79^{b}	96^{ab}	104^{a}	97^{ab}	6.1

[1] C, crimson clover; CL, crimson clover and white mixture; Con., Control; L, white clover.

[2] Standard error of the mean.

[3] dissolved organic carbon

[4] dissolved total nitrogen

[5] dissolved organic nitrogen

[6] microbial biomass as µg C g^{-1}

[7] microbial biomass as µg N g^{-1}

[8] carbon to nitrogen biomass ratio

[9] dehydrogenase enzyme activity, triphenylformazan

[10] N-acetyl-β-D-glucosaminidase enzyme activity

[11] β-glucosidase enzyme activity

[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 6. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by annual legume species in 2-ha paddocks during the 2008 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

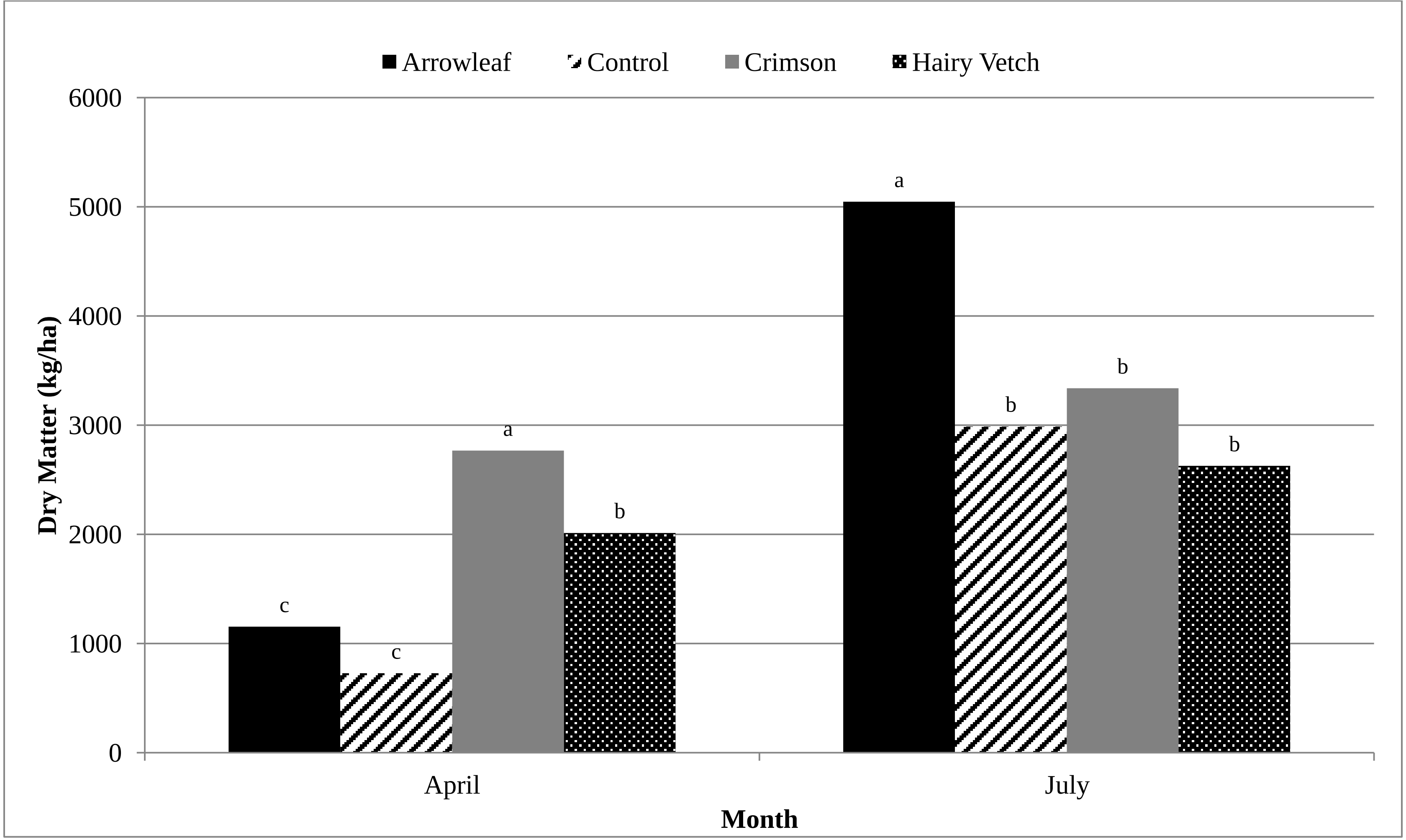
	Treatment				
Species	Crimson	Arrowleaf	Hairy Vetch	Control	SEM[1]
	Frequency of occurrence (%)				
March					
Legume	93.2^{a}	57.2^{b}	95.8^{a}	0.0^{c}	4.52
Bermuda	0.0	0.0	0.0	0.0	0.00
OP[2]	65.3^{b}	97.5^{a}	48.7^{b}	98.6^{a}	7.75
April					
Legume	90.0^{a}	61.8^{b}	98.6^{a}	0.0^{c}	4.75
Bermuda	0.0	0.6	0.4	0.0	0.35
OP	36.8^{b}	79.6^{a}	20.3^{b}	98.0^{a}	7.05
July					
Legume	0.0^{b}	84.8^{a}	1.7^{b}	0.0^{b}	2.88
Bermuda	87.7^{a}	33.0^{b}	85.5^{a}	90.1^{a}	6.86
OP	26.1^{b}	4.3^{c}	20.3^{bc}	46.8^{a}	6.82
August					
Legume	0.0	0.0	0.0	0.0	0.00
Bermuda	94.6^{a}	81.2^{b}	96.6^{a}	88.5^{ab}	4.02
OP	16.5^{b}	11.8^{b}	15.2^{b}	35.3^{a}	5.58
November					
Legume	90.3^{a}	89.3^{a}	91.6^{a}	0.0^{b}	3.41
Bermuda	58.0^{bc}	44.0^{c}	86.2^{a}	84.2^{ab}	10.59
OP	7.7^{b}	6.7^{b}	25.8^{ab}	46.9^{a}	9.10

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category "other".

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 2. Experiment 2: Forage mass in the annual legume species study at the beginning of each grazing cycle during 2008. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed ($P < 0.05$), data is shown for species differences within each month only.



^{a,b,c} Means within a month with unlike superscripts differ ($P < 0.05$).

Table 7. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by annual legume species in 2-ha paddocks during the 2009 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

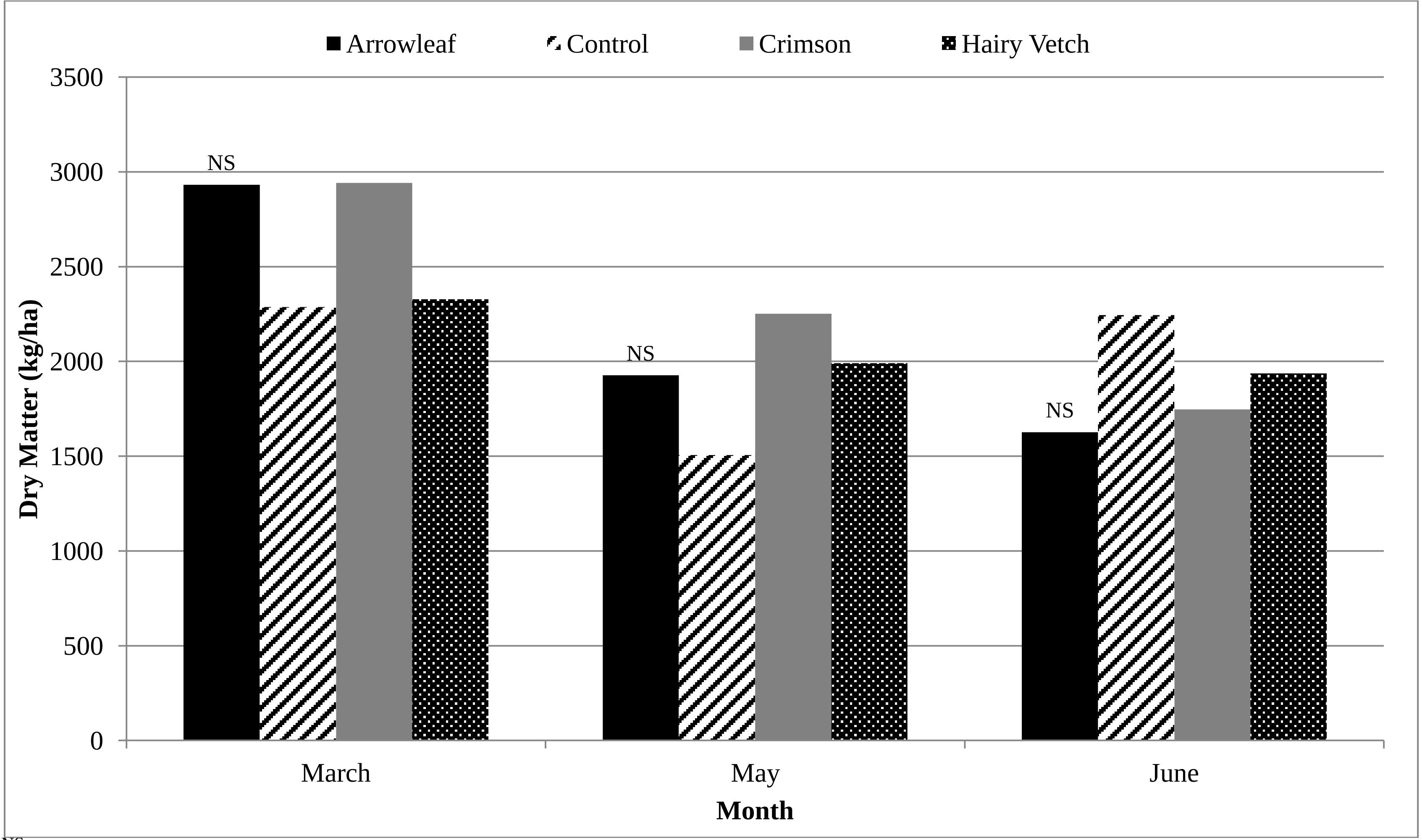
	Treatment				
Species	Crimson	Arrowleaf	Hairy Vetch	Control	SEM[1]
	Frequency of occurrence (%)				
March					
Legume	85.5^{b}	93.2^{ab}	100.0^{a}	0.0^{c}	3.49
Bermuda	0.0	0.0	0.0	0.0	0.00
OP[2]	77.2^{b}	34.7^{c}	25.5^{c}	100.0^{a}	7.35
May					
Legume	78.8^{a}	91.3^{a}	72.8^{a}	0.0^{b}	10.00
Bermuda	47.1	25.2	33.0	52.6	14.44
OP	40.3^{b}	17.1^{c}	25.3^{bc}	100.0^{a}	7.50
June					
Legume	8.5^{b}	48.9^{a}	1.6^{b}	0.0^{b}	4.90
Bermuda	98.3	98.8	99.8	97.8	1.21
OP	34.8^{bc}	24.6^{c}	49.9^{ab}	65.7^{a}	7.83
November					
Legume	6.7^{b}	1.7^{b}	18.6^{a}	0.0^{b}	3.40
Bermuda	98.8	100.0	98.0	87.0	5.04
OP	6.7^{ab}	8.6^{ab}	4.6^{b}	23.8^{a}	7.10

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category “other”.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 3. Experiment 2: Forage mass in the annual legume species study at the beginning of each grazing cycle during 2009. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed ($P < 0.05$), data is shown for species differences within each month only.



^{NS}Means within a month without superscripts are not significantly different ($P < 0.05$).

Table 8. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by annual legume species in 2-ha paddocks during the 2010 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

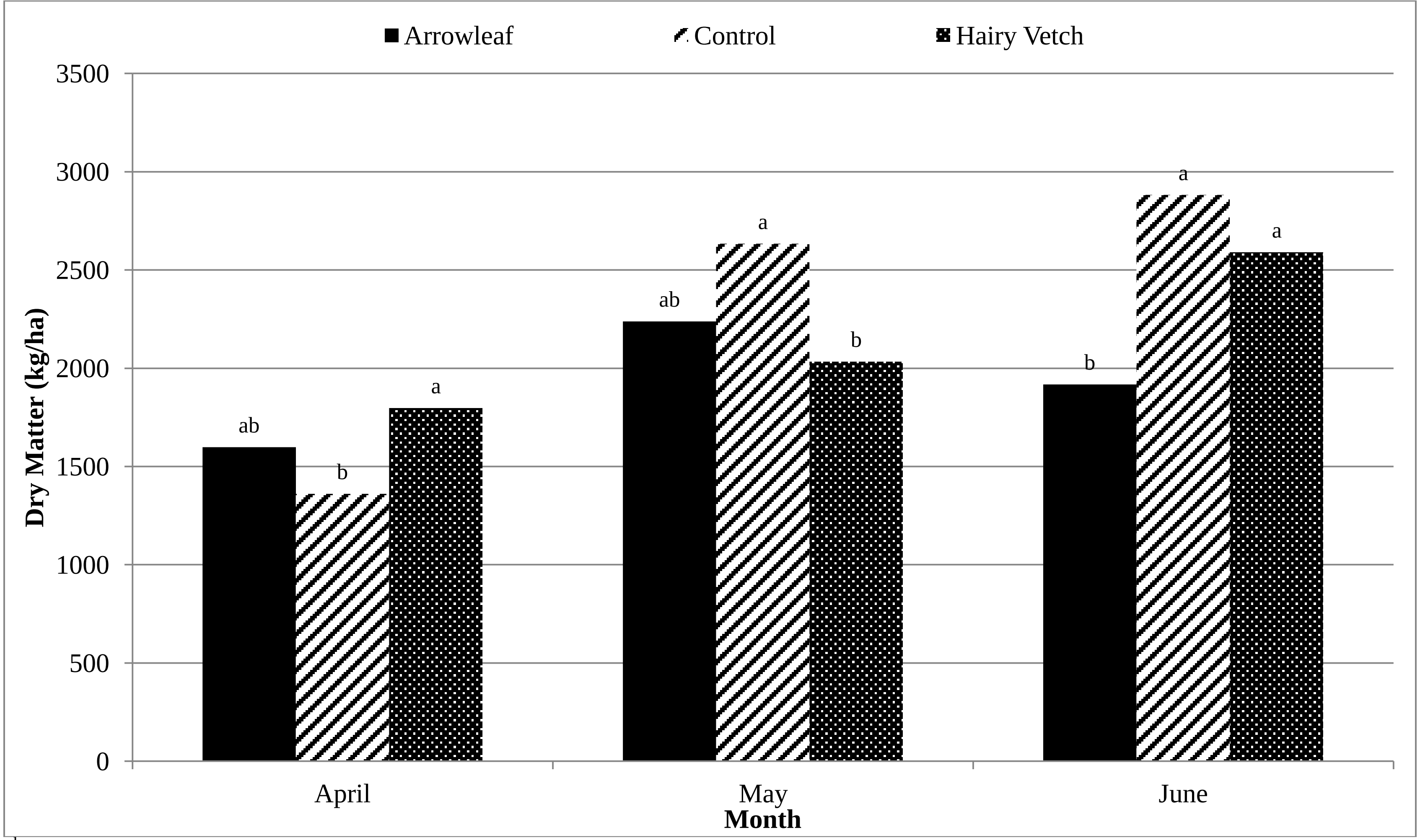
	Treatment			
Species	Arrowleaf	Hairy Vetch	Control	SEM[1]
	Frequency of occurrence (%)			
April				
Legume	45.5^{b}	98.6^{a}	0.0^{c}	7.96
Bermuda	8.2	2.2	1.2	3.45
OP[2]	97.5^{a}	74.2^{b}	100.0^{a}	2.27
May				
Legume	76.2^{b}	99.6^{a}	0.0^{c}	3.74
Bermuda	100.0^{a}	97.6^{ab}	91.8^{b}	2.56
OP	62.1^{b}	1.2^{c}	99.1^{a}	4.38
June				
Legume	42.9^{a}	0.0^{b}	0.0^{b}	7.19
Bermuda	98.2	100.0	99.7	1.05
OP	33.6^{b}	19.2^{b}	67.7^{a}	11.88
October				
Legume	1.2^{ab}	4.2^{a}	0.0^{b}	1.00
Bermuda	100.0	100.0	100.0	0.00
OP	47.3^{a}	25.4^{b}	41.6^{ab}	9.31

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category “other”.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 4. Experiment 2: Forage mass in the annual legume species study at the beginning of each grazing cycle during 2010. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed ($P < 0.05$), data is shown for species differences within each month only.



^{a,b} Means within a month with unlike superscripts differ ($P < 0.05$).

Table 9. Experiment 2: Forage chemical composition parameters as affected by rotationally grazed annual legume species in year 2008. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

	Treatment				
Item[1]	Crimson	Arrowleaf	Hairy Vetch	Control	SEM[2]
April					
NDF[3] (%)	38.8^{b}	40.0^{b}	42.4^{b}	53.1^{a}	2.37
ADF[4] (%)	22.5^{b}	22.1^{b}	27.2^{a}	27.7^{a}	1.17
ADL[5] (%)	3.2^{b}	3.0^{b}	5.0^{a}	5.2^{a}	0.41
N (%)	3.4^{b}	3.0^{b}	4.1^{a}	2.4^{c}	0.18
July					
NDF (%)	70.1^{a}	61.8^{b}	68.8^{a}	68.8^{a}	1.00
ADF (%)	41.2^{a}	39.9^{a}	40.1^{a}	33.4^{b}	1.09
ADL (%)	9.2^{a}	8.8^{a}	9.5^{a}	5.9^{b}	0.68
N (%)	2.5^{b}	2.3^{c}	3.0^{a}	2.3^{c}	0.09

[1] All items reported are on a DM basis.

[2] Standard error of the mean.

[3] Neutral detergent fiber.

[4] Acid detergent fiber.

[5] Acid detergent lignin.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 10. Experiment 2: Forage chemical composition parameters as affected by rotationally grazed annual legume species in year 2009. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

Item[1]	Treatment				SEM[2]
	Crimson	Arrowleaf	Hairy Vetch	Control	
March					
NDF[3] (%)	40.2^{b}	37.7^{b}	45.7^{ab}	52.2^{a}	2.94
ADF[4] (%)	22.2^{b}	21.4^{b}	27.3^{a}	26.3^{a}	1.43
ADL[5] (%)	3.4^{b}	3.3^{b}	5.4^{a}	4.2^{ab}	0.48
N (%)	3.9^{a}	4.1^{a}	4.4^{a}	2.7^{b}	0.39
May					
NDF (%)	51.7^{b}	41.0^{c}	53.1^{b}	62.2^{a}	2.38
ADF (%)	40.0^{a}	32.7^{b}	41.0^{a}	33.1^{b}	1.92
ADL (%)	7.1^{b}	3.5^{c}	10.6^{a}	4.9^{c}	0.56
N (%)	3.1^{c}	4.0^{b}	4.5^{a}	2.7^{d}	0.17
June					
NDF (%)	67.5^{a}	55.0^{b}	66.7^{a}	65.7^{a}	1.98
ADF (%)	30.8	27.0	28.5	30.4	1.38
ADL (%)	5.0^{a}	4.0^{ab}	3.8^{b}	4.3^{ab}	0.42
N (%)	3.0^{a}	2.8^{a}	3.0^{a}	2.3^{b}	0.21

[1] All items reported are on a DM basis.

[2] Standard error of the mean.

[3] Neutral detergent fiber.

[4] Acid detergent fiber.

[5] Acid detergent lignin.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 11. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by perennial legume species in 2-ha paddocks during the 2008 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$). Values do not necessarily add up to 100, as all three types of plants/vegetation have occurred repeatedly within the frequency grid. In addition, only plants with photosynthetically active tissue were counted. Therefore, in November, bermudagrass was mostly dormant already as reflected by low values.

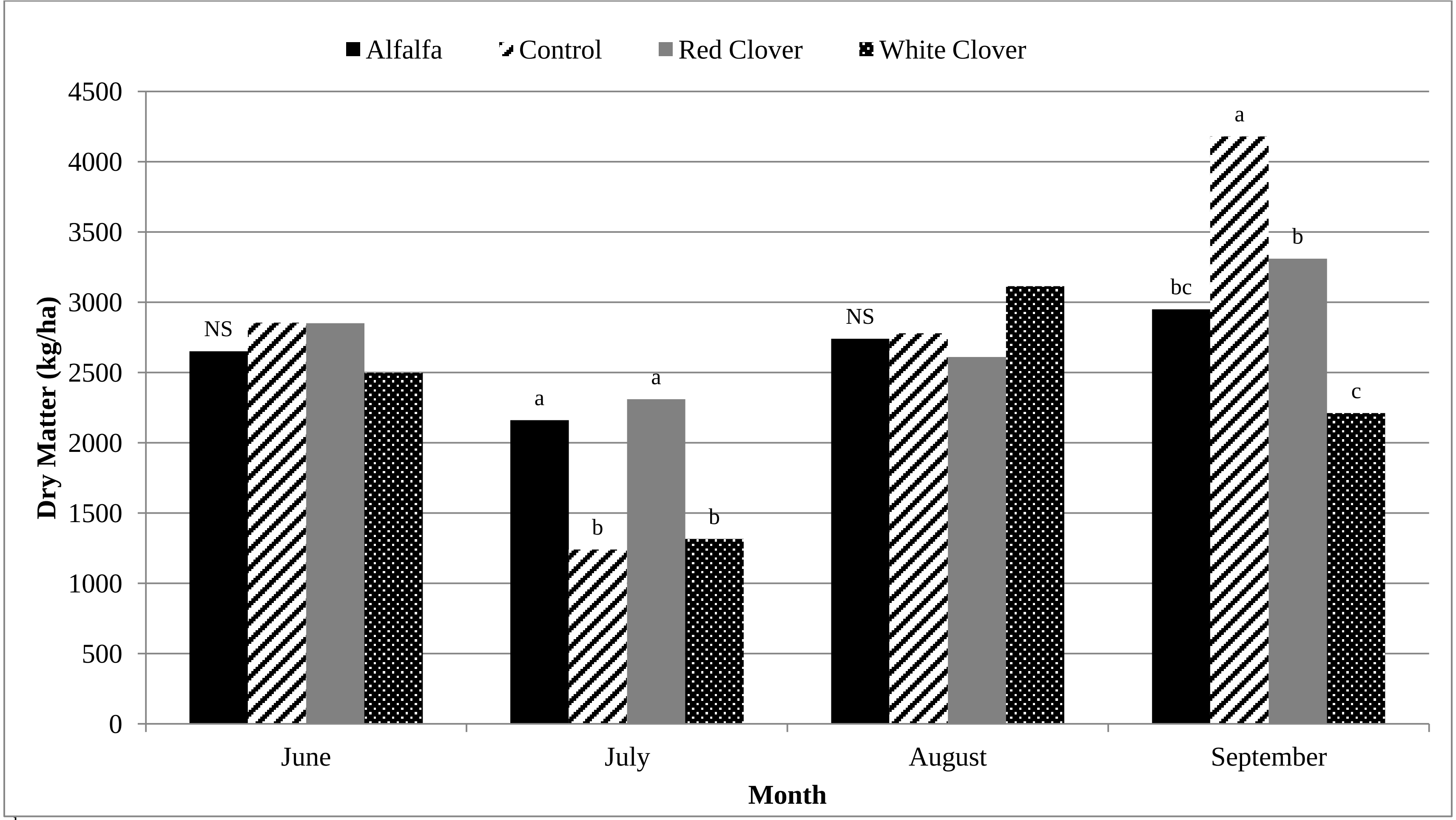
	Treatment				
Species	White Clover	Red Clover	Alfalfa	Control	SEM[1]
	Frequency of occurrence (%)				
April					
Legume	25.7^{a}	39.4^{a}	19.7^{ab}	0.0^{b}	9.70
Bermuda	0.0	0.0	0.0	0.0	0.00
OP[2]	97.9	99.9	99.4	99.5	1.04
June					
Legume	27.1^{ab}	31.4^{a}	28.0^{a}	0.0^{b}	15.82
Bermuda	66.4^{ab}	54.8^{b}	52.9^{b}	79.1^{a}	8.16
OP	57.1^{ab}	36.1^{b}	61.0^{ab}	83.7^{a}	12.41
July					
Legume	49.3^{a}	76.1^{a}	73.3^{a}	0.0^{b}	10.57
Bermuda	75.9^{ab}	68.8^{ab}	58.8^{b}	91.2^{a}	8.36
OP	21.2^{ab}	10.0^{b}	42.1^{a}	40.7^{a}	7.60
August					
Legume	72.2^{ab}	86.3^{a}	48.6^{b}	0.0^{c}	8.68
Bermuda	89.0^{ab}	74.5^{b}	78.0^{b}	96.7^{a}	5.49
OP	37.7^{b}	10.8^{c}	59.3^{a}	68.1^{a}	7.90
September					
Legume	81.8^{a}	84.7^{a}	26.3^{b}	0.0^{b}	9.76
Bermuda	60.8^{bc}	47.3^{c}	79.1^{ab}	91.7^{a}	10.80
OP	26.3^{b}	19.0^{b}	70.6^{a}	68.0^{a}	9.90
November					
Legume	86.0^{a}	88.2^{a}	26.6^{b}	0.0^{c}	6.93
Bermuda	26.9	33.3	46.3	39.6	9.85
OP	1.6^{b}	12.4^{ab}	31.3^{a}	7.9^{ab}	7.98

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category “other”.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 5. Experiment 2: Forage mass in the perennial legume species study at the beginning of each grazing cycle during 2008. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed (P < 0.05), data is shown for species differences within each month only.



^{a,b,c} Means within a month with unlike superscripts differ (P < 0.05).

^{NS} Means within a month without superscripts are not significantly different (P > 0.05).

Table 12. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by perennial legume species in 2-ha paddocks during the 2009 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

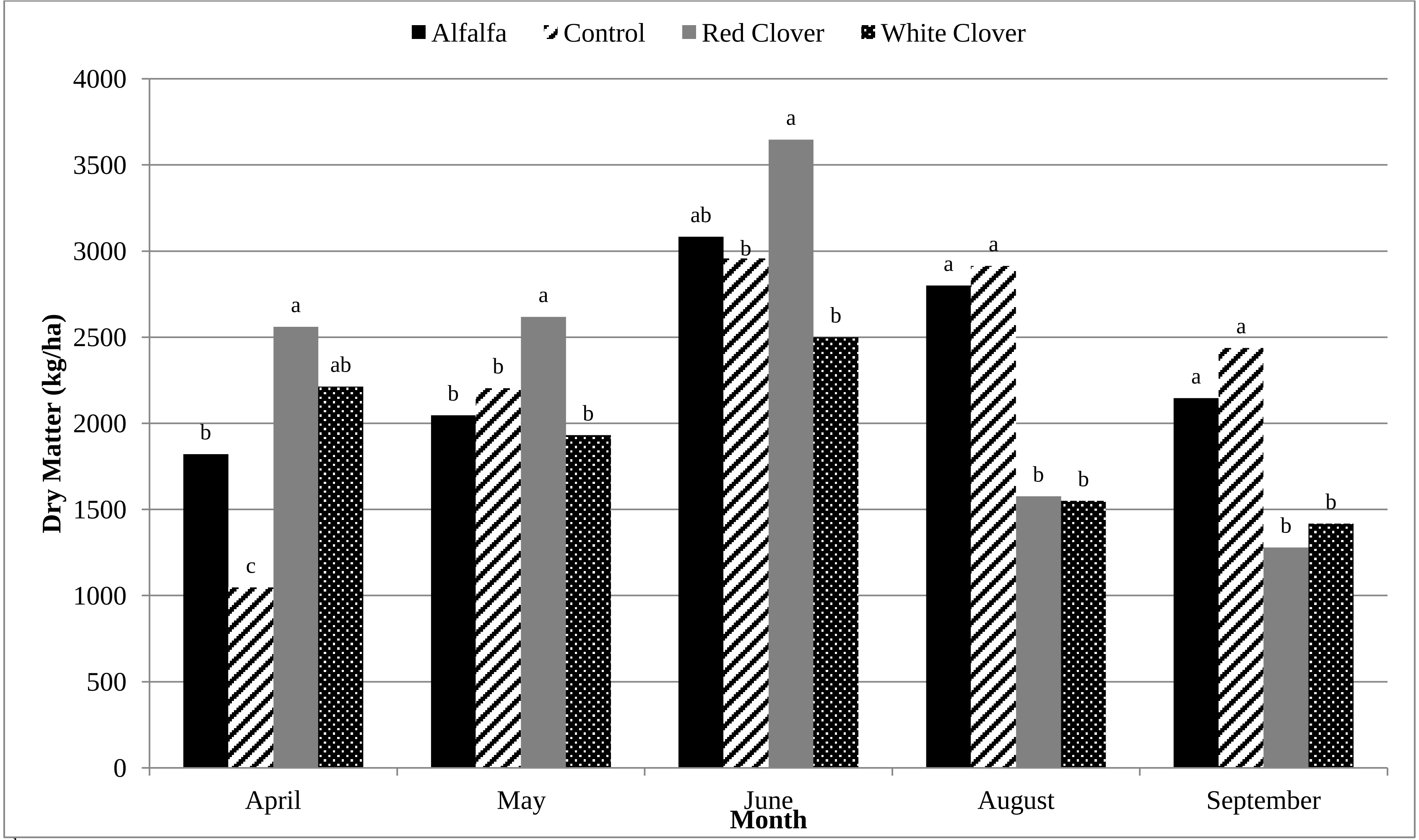
	Treatment				
Species	White Clover	Red Clover	Alfalfa	Control	SEM[1]
	Frequency of occurrence (%)				
March					
Legume	83.4^{a}	89.7^{a}	29.8^{b}	0.0^{c}	8.46
Bermuda	0.0	0.0	0.0	0.0	0.00
OP[2]	44.4^{b}	82.1^{a}	88.8^{a}	99.9^{a}	7.48
April					
Legume	87.6^{a}	91.8^{a}	23.2^{b}	0.0^{c}	7.51
Bermuda	0.0	0.0	0.0	0.0	0.00
OP	44.8^{c}	64.4^{b}	100.0^{a}	100.0^{a}	6.79
May					
Legume	94.4^{a}	97.0^{a}	27.1^{b}	0.0^{c}	5.52
Bermuda	21.0^{c}	8.0^{c}	49.0^{b}	100.0^{a}	9.93
OP	23.4^{b}	45.9^{b}	94.7^{a}	100.0^{a}	8.17
June					
Legume	96.8^{a}	95.7^{a}	55.2^{b}	0.0^{c}	10.34
Bermuda	65.7^{ab}	43.6^{b}	85.6^{a}	88.7^{a}	7.81
OP	21.8^{b}	7.6^{b}	64.3^{a}	58.2^{a}	8.85
August					
Legume	93.3^{a}	87.5^{a}	27.3^{b}	0.0^{c}	5.73
Bermuda	73.4^{b}	64.4^{b}	97.4^{a}	95.4^{a}	4.93
OP	39.0^{b}	23.7^{b}	77.1^{a}	78.3^{a}	7.02
September					
Legume	95.4^{a}	79.8^{b}	15.4^{c}	0.0^{c}	5.13
Bermuda	85.1^{ab}	79.7^{b}	97.9^{a}	95.0^{a}	5.38
OP	31.4^{b}	30.0^{b}	80.6^{a}	88.7^{a}	7.11
November					
Legume	94.0^{a}	67.5^{b}	3.0^{c}	0.0^{c}	5.01
Bermuda	75.3^{b}	95.0^{a}	100.0^{a}	99.9^{a}	5.09
OP	1.7^{b}	24.7^{ab}	39.3^{a}	40.0^{a}	10.23

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category “other”.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 6. Experiment 2: Forage mass in the perennial legume species study at the beginning of each grazing cycle during 2009. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed ($P < 0.05$), data is shown for species differences within each month only.



^{a,b,c} Means within a month with unlike superscripts differ ($P < 0.05$).

Table 13. Experiment 2: Frequency of occurrence of legumes, bermudagrass, and other plants during spring and summer as affected by perennial legume species in 2-ha paddocks during the 2010 growing season. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

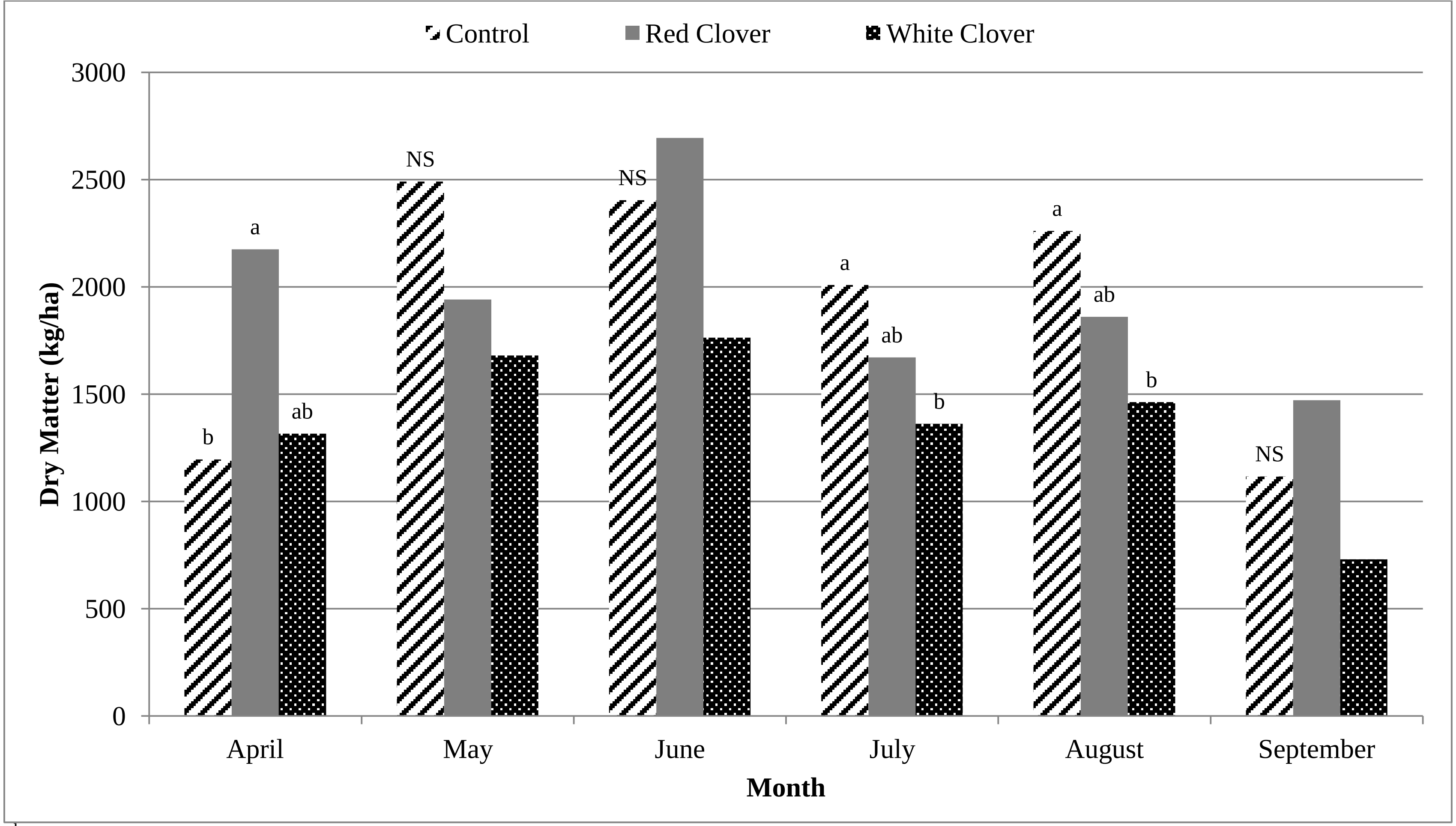
Species	Treatment			SEM[1]
	White Clover	Red Clover	Control	
	Frequency of occurrence (%)			
April				
Legume	97.2^{a}	88.1^{a}	0.0^{b}	3.18
Bermuda	0.2	0.2	0.5	0.23
OP[2]	52.7^{b}	92.8^{a}	100.0^{a}	7.07
May				
Legume	96.4^{a}	88.2^{a}	0.0^{b}	3.86
Bermuda	77.0	72.3	100.0	10.35
OP	17.5^{b}	27.1^{b}	100.0^{a}	9.33
June				
Legume	96.9^{a}	93.5^{a}	0.0^{b}	2.51
Bermuda	94.2^{a}	71.9^{b}	99.0^{a}	6.86
OP	29.9^{b}	28.7^{b}	100.0^{a}	6.13
July				
Legume	59.3^{a}	48.4^{a}	0.0^{b}	8.33
Bermuda	90.6	93.1	97.2	3.92
OP	54.4^{ab}	29.6^{b}	75.5^{a}	11.35
August				
Legume	28.6^{a}	17.1^{ab}	0.0^{b}	6.67
Bermuda	93.7	97.6	99.0	2.48
OP	48.0^{ab}	25.8^{b}	71.1^{a}	10.5
October				
Legume	44.0^{a}	12.5^{b}	0.0^{b}	6.74
Bermuda	97.9	99.7	100.0	1.22
OP	53.1	28.4	57.1	9.64

[1] Standard error of the mean.

[2] Species that were not identified as bermudagrass, ryegrass, or the appropriate legume for the treatment being assessed were counted for category “other”.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Figure 7. Experiment 2: Forage mass in the perennial legume species study at the beginning of each grazing cycle during 2010. Data are presented for each month in which grazing began. Although a treatment by month interaction was observed ($P < 0.05$), data is shown for species differences within each month only.



^{a,b} Means within a month with unlike superscripts differ ($P < 0.05$).
^{NS} Means within a month without superscripts are not significantly different ($P > 0.05$).

Table 14. Experiment 2: Forage chemical composition parameters as affected by rotationally grazed perennial legume species in year 2008. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

Item[1]	Treatment				SEM[2]
	White Clover	Red Clover	Alfalfa	Control	
June					
NDF[3] (%)	52.4^{b}	53.4^{b}	51.5^{b}	60.9^{a}	1.74
ADF[4] (%)	32.9	29.8	33.7	33.2	1.58
ADL[5] (%)	6.8^{bc}	5.8^{c}	7.4^{ab}	8.4^{a}	0.52
N (%)	3.2^{a}	2.7^{ab}	3.0^{a}	2.5^{b}	0.16
July					
NDF (%)	62.1^{a}	48.7^{b}	56.5^{b}	68.1^{a}	3.15
ADF (%)	34.4^{a}	29.8^{b}	32.7^{ab}	33.7^{ab}	1.77
ADL (%)	6.7^{a}	5.2^{b}	6.4^{ab}	6.1^{ab}	0.60
N (%)	2.3^{b}	2.8^{a}	2.7^{ab}	2.2^{b}	0.16
August					
NDF (%)	68.3^{a}	62.9^{b}	66.7^{a}	67.8^{a}	1.32
ADF (%)	32.1	33.1	32.3	31.8	0.77
ADL (%)	4.8^{ab}	5.2^{a}	4.9^{ab}	3.7^{b}	0.55
N (%)	1.8^{b}	2.8^{a}	1.8^{b}	1.7^{b}	0.10
September					
NDF (%)	57.9^{b}	52.2^{b}	66.4^{a}	71.4^{a}	2.80
ADF (%)	30.7^{b}	30.3^{b}	40.7^{a}	35.8^{ab}	2.80
ADL (%)	4.3^{b}	5.8^{a}	5.3^{ab}	5.1^{ab}	0.42
N (%)	2.7^{ab}	3.1^{a}	2.3^{b}	1.7^{c}	0.18

[1] All items reported are on a DM basis.

[2] Standard error of the mean.

[3] Neutral detergent fiber.

[4] Acid detergent fiber.

[5] Acid detergent lignin.

[a,b,c] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 15. Experiment 2: Forage chemical composition parameters as affected by rotationally grazed perennial legume species in year 2009. Data are reported monthly due to a month by treatment interaction ($P < 0.05$).

Item[1]	Treatment				SEM[2]
	White Clover	Red Clover	Alfalfa	Control	
May					
NDF[3] (%)	41.1^{c}	41.5^{c}	53.8^{b}	61.4^{a}	1.90
ADF[4] (%)	23.5^{c}	25.4^{c}	30.1^{b}	33.8^{a}	0.95
ADL[5] (%)	3.8^{b}	5.1^{a}	5.2^{a}	5.4^{a}	0.25
N (%)	3.7^{a}	3.6^{a}	3.1^{a}	2.2^{b}	0.27
June					
NDF (%)	40.2^{d}	46.6^{c}	54.7^{b}	66.3^{a}	1.86
ADF (%)	25.6^{b}	36.3^{ab}	30.7^{b}	48.8^{a}	4.38
ADL (%)	4.8	4.9	5.5	5.9	0.61
N (%)	3.8^{a}	3.3^{b}	2.8^{c}	2.0^{d}	0.15
August					
NDF (%)	51.2^{c}	56.2^{bc}	65.2^{ab}	67.7^{a}	3.04
ADF (%)	23.6^{b}	29.9^{a}	30.4^{a}	30.7^{a}	0.85
ADL (%)	3.3^{c}	5.3^{a}	4.4^{b}	4.2^{b}	0.31
N (%)	3.5^{a}	3.1^{ab}	2.4^{b}	2.3^{b}	0.29

[1] All items reported are on a DM basis.

[2] Standard error of the mean.

[3] Neutral detergent fiber.

[4] Acid detergent fiber.

[5] Acid detergent lignin.

[a,b,c,d] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 16. Experiment 2: Soil quality parameters across fall, spring, and summer as affected by over-seeded annual legumes.

	Annuals				
Item	Crimson	Arrowleaf	Hairy Vetch	Control	SEM[1]
Gravimetric Soil Moisture, ($g\ g^{-1}$)	0.24	0.23	0.23	0.24	0.008
Organic Matter (%)	5.71^{a}	5.37^{ab}	5.11^{b}	5.25^{ab}	0.194
DOC[2] ($\mu gC\ g^{-1}$)	88.8^{a}	69.7^{b}	71.7^{ab}	71.3^{ab}	6.26
DTN[3] ($\mu gN\ g^{-1}$)	35.1	29.5	31.1	30.5	2.12
NO_3 ($\mu gN\ g^{-1}$)	7.4	8.7	8.4	8.0	1.06
NH_4 ($\mu gN\ g^{-1}$)	9.1^{a}	5.3^{b}	6.7^{ab}	6.9^{ab}	1.07
Inorganic N ($\mu gN\ g^{-1}$)	16.5	14.0	15.1	14.8	1.30
DON[4]($\mu gN\ g^{-1}$)	18.6^{a}	15.6^{b}	15.9^{ab}	15.7^{ab}	1.01
Microbial Biomass C[5] ($\mu gC\ g^{-1}$)	241^{a}	201^{ab}	197^{b}	220^{ab}	14.2
Microbial Biomass N[6] ($\mu gN\ g^{-1}$)	42.3	37.2	37.1	40.6	2.53
Microbial Biomass C/N[7]	6.05	5.62	5.51	5.55	0.238
DHase[8] ($\mu gTPF\ g^{-1}\ 24\ hr^{-1}$)	471^{a}	376^{b}	341^{b}	409^{ab}	28.7
NAGase[9] (μg p-nitrophenol. $g^{-1}\ hr^{-1}$)	79.0^{a}	69.1^{ab}	62.1^{b}	69.6^{ab}	5.69
PNGase[10] (μg p-nitrophenol $g^{-1}\ hr^{-1}$)	154^{a}	135^{ab}	125^{b}	124^{b}	7.8

[1] Standard error of the mean.

[2] dissolved organic carbon

[3] dissolved total nitrogen

[4] dissolved organic nitrogen

[5] microbial biomass as $\mu g\ C\ g^{-1}$

[6] microbial biomass as $\mu g\ N\ g^{-1}$

[7] carbon to nitrogen biomass ratio

[8] dehydrogenase enzyme activity

[9] N-acetyl-β-D-glucosaminidase enzyme activity

[10] β-glucosidase enzyme activity

[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 17. Experiment 2: Soil quality parameters across fall, spring, and summer as affected by over-seeded perennial legumes.

Item	Perennials				SEM[1]
	White Clover	Red Clover	Alfalfa	Control	
Gravimetric Soil Moisture, (g g^{-1})	0.27	0.27	0.30	0.27	0.012
Organic Matter (%)	5.68^{b}	5.72^{b}	6.25^{a}	5.28^{b}	0.168
DOC[2] (µgC g^{-1})	88.4^{ab}	83.4^{ab}	91.7^{a}	71.9^{b}	6.65
DTN[3] (µgN g^{-1})	39.2^{ab}	34.5^{ab}	41.4^{a}	30.9^{b}	2.87
NO_3 (µgN g^{-1})	9.9	7.9	10.2	7.3	1.14
NH_4 (µgN g^{-1})	8.3	7.7	11.7	6.7	2.22
Inorganic N (µgN g^{-1})	18.3^{ab}	15.6^{ab}	21.9^{a}	14.0^{b}	2.26
DON[4](µgN g^{-1})	20.9^{a}	18.9^{ab}	19.6^{ab}	16.9^{b}	1.01
Microbial Biomass C[5] (µgC g^{-1})	239^{ab}	232^{ab}	256^{a}	208^{b}	12.4
Microbial Biomass N[6] (µgN g^{-1})	48.2^{ab}	46.0^{ab}	53.5^{a}	42.6^{b}	3.30
Microbial Biomass C/N[7]	4.93	5.12	4.83	5.01	0.257
DHase[8] (µgTPF g^{-1} 24 hr^{-1})	492^{ab}	461^{ab}	529^{a}	435^{b}	28.9
NAGase[9] (µg p-nitrophenol. g^{-1} hr^{-1})	81.1	86.7	89.9	76.2	5.69
PNGase[10] (µg p-nitrophenol g^{-1} hr^{-1})	153^{a}	152^{a}	160^{a}	120^{b}	7.1

[1] Standard error of the mean.
[2] dissolved organic carbon
[3] dissolved total nitrogen
[4] dissolved organic nitrogen
[5] microbial biomass as µg C g^{-1}
[6] microbial biomass as µg N g^{-1}
[7] carbon to nitrogen biomass ratio
[8] dehydrogenase enzyme activity
[9] N-acetyl-β-D-glucosaminidase enzyme activity
[10] β-glucosidase enzyme activity
[a,b] Means within a row with unlike superscripts differ ($P < 0.05$). Means within a row without superscripts are not significantly different ($P > 0.05$).

Table 18. Experiment 2: Seeding rates for annual and perennial legume species.

	Perennials			Annuals		
	White Clover	Red Clover	Alfalfa	Crimson Clover	Arrowleaf Clover	Hairy Vetch
Seeding Rate (kg ha^{-1})	6	17	27	38	13	32