ABSTRACT

PEHIM LIMBU, SMRITI. Fall Stockpiled Tall Fescue Response to Nitrogen Fertilization as Affected by Soil Biological Quality. (Under the direction of Dr. Alan Franzluebbers)

Application of nitrogen (N) fertilization prior to stockpiling is a common practice to promote herbage accumulation. However, there are no soil tests to accurately predict the likelihood of yield response of tall fescue to N fertilization. Soil biological activity contributes to N mineralization, which could be a source of N to plants during the growing season. Pastures with a diversity of management (22 in 2015 and 35 in 2016) were tested in North Carolina, Virginia, West Virginia, and Georgia. Each field had four N treatments (0, 45, 90, 135 kg N ha^{-1}) replicated four times in a randomized complete block design (16 plots, 3×6 m each). Urea fertilizer was applied at the beginning of September 2015 and 2016. Forage was allowed to grow until harsh winter conditions set in (December to January), at which time plots were mechanically harvested to determine dry matter (DM) production. Harvest heights were $\geq$10 cm in 2015 and $\geq$10 cm and 5-10 cm in 2016. Soil was sampled at 0-10 cm depth in August/September of each year. Soil N supplying capacity was assessed with net N mineralization during aerobic incubation at 25^0C for 24 days. Other soil biological assessments were total N and carbon (C), soil microbial biomass C (SMBC), particulate organic C and N, and the flush of CO_2. Soil N supplying capacity was also assessed with greenhouse growth trials of sorghum-sudangrass during 6 weeks. Two separate greenhouse trials were conducted in June/July 2016 and February/March 2017.

Relative yield response at threshold yield efficiency of 5 kg DM kg^{-1} N was highly correlated with the flush of CO_2 ($r^2 = 0.86$), cumulative C mineralization ($r^2 = 0.76$), net N mineralization ($r^2 = 0.80$), and SMBC ($r^2 = 0.60$), as well as at threshold yield efficiency of

10 kg DM kg^{-1} N (r^2 = 0.86, 0.73, 0.81 and 0.59, respectively). Similarly, the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC were highly correlated with economically optimum N rate at threshold yield efficiencies of 5 kg DM per kg^{-1} N (r^2 = 0.63, 0.53, 0.60, and 0.53 respectively) and 10 kg DM kg^{-1} N (r^2 = 0.74, 0.63, 0.70, and 0.53 respectively). There was a weaker correlation between soil biological quality measures and yield response at harvest height of 5-10 cm. Flush of CO_2 was determined to be the best predictor of yield response of stockpiled tall fescue to N fertilization.

Crude protein and moisture content of stockpiled tall fescue were significantly related with N fertilizer rate, but not to soil biological properties. Both crude protein and moisture content were greater for $\geq$10 cm harvest height than those for 5-10 cm height.

In greenhouse growth assays, plant DM correlated strongly with the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC in Trial 1 (r^2= 0.66, 0.59, 0.62, and 0.58, respectively) and Trial 2 (r^2 = 0.56, 0.63, 0.64, and 0.55, respectively). Similarly, plant N uptake also correlated with the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC in Trial 1 (r^2 = 0.77, 0.72, 0.58, and 0.70, respectively) and Trial 2 (r^2 = 0.72, 0.81, 0.68, and 0.37, respectively).

Clearly, soil biological activity influences yield response of fall stockpiled tall fescue to N fertilization. Various indicators of soil biological activity (including the short-term assay of the flush of CO_2) were good predictors of plant DM production and N uptake in a semi-controlled environment, and of relative yield response to N fertilizer in field studies. Soil biological indicators should be used to differentiate non-responsive and responsive fields to N fertilization. This approach will help producers and the environment.

Fall Stockpiled Tall Fescue Response to Nitrogen Fertilization as Affected by Soil Biological Quality

by
Smriti Pehim Limbu

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APPROVED BY:

Dr. Alan J. Franzluebbers Committee Chair	Dr. Matthew H. Poore

Dr. Miguel S. Castillo

DEDICATION

To mom and dad.

BIOGRAPHY

Smriti was born in Nepal. She studied science, applied mathematics and literature in her high school and joined Institute of Agriculture and Animal Science, Chitwan for her undergraduate studies in Agricultural Science in 2010. She worked for Nepal Ministry of Agricultural Development for a while then, she joined NCSU for her master’s degree in August 2015. Her goal in life is to contribute towards sustainable agriculture and social reforms.

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INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Tall fescue is the most important temperate perennial grass in the world (Hoveland, 2009). Wide adaptation, hardiness, high nutritive value throughout the year and low maintenance requirements of this forage has made it widely popular in the eastern USA. One of the management strategies in tall fescue pastures is stockpiling. Stockpiling is the practice of strategic accumulation of forage growth for later use. In the eastern region, fall stockpiling and winter grazing reduces cost of winter feed and can minimize ergot alkaloid exposure to grazing ruminants, which otherwise is present in wild-type endophyte-infected tall fescue forages.

A key limiting factor in most forage systems is N, and is considered important in fall stockpiled tall fescue (Teutsch et al., 2005). In the southeastern USA, soils are generally low in organic matter (Sikora and Moore-Kucera, 2014) and therefore, there is need for N to obtain satisfactory herbage accumulation. However, rate, timing and source of N fertilization can have an effect on yield and quality of stockpiled tall fescue pastures. Along with various soil properties, the N cycle in pastures has to be understood to make effective N recommendations.

This review will: (i) introduce tall fescue as one of the most important forage crops in the eastern USA; (ii) elaborate on the importance of stockpiling of tall fescue; (iii) discuss the different sources of N fertilization in the eastern USA along with the advantages and disadvantages of different rate and timing of fertilization; and (iv) examine different soil properties as influential factors for yield of tall fescue and its response to N fertilization.

LITERATURE REVIEW

Origin and distribution of tall fescue

Tall fescue (*Festuca arundinacea*; syn, *Schedonorus arundinaceus* and *Lolium arundinaceum*), a native of Europe, is a cool season perennial grass. Due to its adaptation to a wide range of climatic and edaphic factors (Niazkhani et al., 2014) and excellent agronomic features (Lou et al., 2015), tall fescue is one of the most widely grown, temperate perennial grasses in the world (Hoveland, 2009). Tall fescue has a high level of persistence compared to other cool season perennial forages. It can withstand pressure from grazing livestock and is tolerant to summer stress (Franzluebbers et al., 2012). These attributes along with other traits like rapid germination, low maintenance requirements and favorable yield potential make tall fescue popular as a forage and turf grass and is also used for land reclamation. Tall fescue pastures are found in cool climates throughout North Africa, West and Central Asia, Western Europe and North America (Niazkhani et al., 2014). Tall fescue was introduced in the USA from Europe in the late 1800s, probably as a contaminant in hay or packing materials (Saikkonen et al., 2016). Now, it is the most widely planted forage grass in the United States (Waller, 2009) with 9.7 million hectares in the eastcentral and southeastern United States feeding more than 8 million beef cows in this region (Teutsch, 2011). Tall fescue is also widespread in the northwestern United States, where it is grown for seed production (Hannaway et al., 2009).

Contribution of endophyte to tall fescue

The wide distribution of tall fescue can be partially attributed to infection of the plant with aboveground, vertically transmitted, seed borne fungal endophytes from the genus *Neotyphodium*, capable of producing a number of metabolites in association with host plants. This fungus grows intracellularly in the leaf sheath (Bacon et al., 1986) and resides primarily within basal stem tissue (Franzluebbers et al., 1999). Several researchers report that tall fescue stands infected with an endophyte (E+) persist better than tall fescue cultivars without an endophyte (E-) under; (i) drought possibly because of lower net photosynthetic rate, higher stomatal resistance (Belesky et al., 1987) and greater root proliferation under drought-stressed condition (Richardson et al., 1990), (ii) pests of grasses like aphids, Argentine stem weevil, larva of army worm and nematode that use tall fescue as a source of food (Kimmons et al., 1990), and (iii) poor soil fertility and intensive grazing (or overgrazing).

Endophyte toxicosis

While the association between endophyte and tall fescue helps in the persistence and vigor of the plant, it can also potentially cause toxicity in livestock consuming it. The endophyte fungus, *Neotyphodium coenophialum*, in association with tall fescue can synthesize ergot alkaloids such as clavinet alkaloids, lysergic acid amides, and ergopeptines (Bacon et., 1977). Ergopeptine alkaloids are considered the primary agent in causing toxicosis (Smith et al., 2012). Ergot alkaloids are present in leaves and stem but are highly concentrated in seed of the plant (Rottinghaus et al., 1991). Tall fescue toxicosis can cause disorders, such as fat necrosis, and fescue foot characterized by lower weight gain, less milk production, lower conception rate and rougher hair coats in animals consuming the infected plant (Stuedemann and Hoveland, 1988; Kallenbach et al., 2003). Stuedemann and Hoveland (1988) reported that cattle grazed or fed tall fescue hay with high endophyte level ingested 65 to 92% as much forage, produced 57 to 83% as much milk, gained 21 to 78% as much weight per day, and gained 65 to 89% as much weight per hectare as compared to animals fed tall fescue with a low level of endophyte. Total losses to producers in the USA due to tall fescue toxicosis has been estimated to be $1 billion annually (Strickland et al., 2011).

Novel endophyte as a strategy to reduce toxicosis

Recently, endophyte strains that do not produce ergot alkaloids, known as novel endophyte have been inserted into tall fescue, thereby alleviating toxicosis while maintaining plant persistence and vigor. For the very first time, a tall fescue cultivar, ‘Jesup’ was infected with a novel endophyte, AR542 and commercially released as Jesup-MaxQ (MaxQ) by AgResearch Ltd., New Zealand (Phillips and Aiken, 2009; Johnson et al., 2012; Aiken and Strickland, 2015). Parish et al. (2003) found that a non-ergot alkaloid producing endophyte like MaxQ is a promising way to alleviate fescue toxicosis. Other novel endophyte infected tall fescue cultivars have also been released after the release of MaxQ. A distinction can be made between wild-endophyte association and novel-endophyte association (Franzluebbers and Hill, 2005). The persistence of tall fescue pastures with novel endophyte has been found to be better than in pastures with endophyte-free stands and nearly similar to wild-type endophyte infected tall fescue during a 6-yr grazing experiment (Franzluebbers et al., 2009). Georgia-5 novel-endophyte tall fescue had only minimal losses in a 4-yr continuous grazing experiment (Alison, 2006). Novel endophytes seem to persist well with careful management but their survival during summer slump is a major concern and good grazing management practices will be needed for novel endophyte fescue pastures to maintain persistence especially while grazing during summer and late spring (Aiken and Strickland, 2015). Also, replacement of wild-endophyte infected tall fescue pastures with novel endophyte can be challenging where there are steep slopes and shallow soils which are prone to erosion and have poor water-holding capacity (Coblentz et al., 2006). Adoption of novel endophyte tall fescue pastures have other challenges of high seed cost, and lack of proper knowledge on the loss due to toxic endophytes on the part of the producers (Poore and Washburn, 2013).

Other strategies to reduce toxicosis

Since the primary means of transmission of the endophyte is seed, use of fungus free seeds for planting by harvesting seeds from fungus-free plants, storing seeds for a year before planting, heat treating infected seeds and chemical treating of seeds can also be used for controlling toxicosis problem. Use of metsulfuron has been found to suppress seed head emergence (Aiken et al., 2012) but suppression of seed head in toxic endophyte tall fescue is also associated with a reduction in forage availability up to 51% (Turner et al., 1990). Alternatively, mixing of fescue stands with legumes has been effectively practiced by producers in many states to dilute the effects of alkaloid ingestion by livestock. And once affected, supplemented diet to promote more rapid weight gain and improved recovery and treatment through the administration of a therapeutic dosage of thiamin has been proposed (Dougherty and Gay, 1988).

Fall stockpiling as a grazing strategy

Stockpiling of tall fescue, a practice of accumulating forage for winter grazing, may be considered another way of reducing toxicosis. In a study by Kallenbach et al. (2003), ergovaline alkaloid concentration declined by 85% in Kentucky-31 E+ fescue from mid-December to the end of winter, which suggests that stockpiled forage became less toxic to animals. Similar results of decline in ergovaline concentration with delayed defoliation from December to February was observed in a 3-year study by Burns et al. (2006). Drewnoski et al. (2007) measured total ergot alkaloids instead of ergovaline alone and found an 81% decrease in ergot alkaloids from December to January.

Profitability is a key factor in making production and management decisions, and researchers over time have found out that grazing stockpiled forages is a more cost effective method of wintering livestock than other options like hay-feeding (Poore and Drewnoski, 2010). Stockpiling of fescue extends the grazing season (Nave et al., 2016). Using stockpiling and grazing instead of a hay-feeding system might also be more environment-friendly and also might be healthier for raising cattle because of a cleaner environment (Poore and Drewnoski, 2010) as intact grass with the soil avoids puddling by animals around hay feeding stations.

Tall fescue is well suited to fall stockpiling for winter grazing because it: (i) produces more autumn growth than other cool season forages (Mays and Wasko, 1960), (ii) retains more nutritive value than other forage crops available for winter feed (Hitz and Russell, 1998), (iii) provides palatable green forage through the winter due to its cold tolerance attributed to its heavy waxy cuticle on leaves and its chemistry that preserves cell function at cold temperature (Jennings et al., 2009) and (iv) shows little effect of winter defoliation in growth the following season (Kallenbach et al., 2003). In fact, the ability of tall fescue to be stockpiled is one of its strongest attributes (Teutsch et al., 2005).

Quality of forage from stockpiling

Factors that influence the success of tall fescue as stockpiled forage are accumulation period, rainfall and fertilization management (Matches and Burns, 1995). Generally, late summer stockpiling of tall fescue provides forage with adequate nutritive value for grazing beef cows (Hitz and Russell, 1998). Poore and Drewnoski (2010) in their review revealed that the nutritive value of stockpiled tall fescue is at or above the requirements of dry cow and in some instances, of a lactating cow with moderate milk production but the requirement of growing cattle may not be met which may be due to low level of dry matter intake. A study of three pastures at the southwest Missouri Research and Education Center near Mt. Vernon, MO by Kallenbach et al. (2003) revealed that stockpiled tall fescue had no decline in herbage mass and only minor decline in nutritive value while stockpiling. Crude protein (CP) declined from mid-December to early March and acid detergent fiber (ADF) and neutral detergent fiber (NDF) increased with time. Others have noted a decline in nutritive value after early winter. Although there were changes in ADF, NDF and CP, the nutritive value of the stockpiled forage met or exceeded the nutritional requirements of both dry and lactating beef cow (Kallenbach et al., 2003). Also, the stockpiled tall fescue in this study had less ADF and NDF as compared to the fully headed tall fescue hay that is generally fed to livestock during winter in that area.

Need of nitrogen in the southeastern USA

A primary factor affecting yield of stockpiled tall fescue is N availability (Teutsch et al., 2005), as N is often the most limiting nutrient in pasture. Adding N to tall fescue may be important for maximum yield and quality even through the winter months. Halich et al. (2014) reported a study conducted in Kentucky that showed greater forage production by over a ton and greater protein by 5 percentage points with N application of 102 kg ha^{-1}. Also, in Ohio, similar N application rate increased protein by 9 percentage points and improved overall digestibility (Halich et al., 2015).

Soils in the southeastern USA are low in soil organic matter levels compared to the northern region of the USA because of the subtropical climate resulting in year-round favorable temperature and moisture for microbial growth and organic matter mineralization (Sikora and Moore-Kucera, 2014). Supplemental N is often considered necessary for optimal productivity of pastures in the southeastern USA.

Rate and timing of nitrogen fertilization

The decision of whether to apply N should be considered by taking weather conditions and especially rainfall into consideration as it affects the N uptake by plants (Nave et al., 2016). Also, year-to-year environmental variation in temperature and rainfall may sometimes have greater influence on yield response than N application (Collins and Balasko, 1981). Greater yield response to N can be expected when N is applied right before rainfall (Riesterer et al., 2000). Late summer N application may not result in greater yields if the soil moisture remains low (Poore et al., 2000).

Teutsch et al. (2005) found that N application rate had no significant effect on forage CP, ADF, NDF and TDN. Although, yield was greater for greater N rates, DM produced per unit of N and maximum yield with timing of N fertilization varied from year to year as a result of different rainfall patterns (Teutsch, 2011). Yield response to N was greater if rainfall followed N fertilization regardless of application time. Poore and Drewnoski (2010) revealed that N utilization efficiency most often falls between 10 and 20 kg DM kg^{-1} N when applied in late summer (August to September), ranging from 7 to 33 kg DM kg^{-1} N applied. Among N application rates studied (0 to 135 kg ha^{-1}), the most effective dose was 45 to 50 kg N ha^{-1} (Poore and Drewnoski, 2010).

In Kentucky, the optimal time to apply N is in early to mid-August as prior applications may encourage the growth of weedy grasses like crabgrass while waiting until September or beyond will reduce N utilization (Halich et al., 2014). Gerrish et al. (1994) mentioned a decrease in yield if stockpiling was initiated after mid-August. According to the review of Poore and Drewnoski (2010), the earlier stockpiling is initiated, the higher the yield response, but the lower the nutritive value during winter, therefore, as a compromise between yield and nutritive value, it is generally recommended to fertilize in August (in more northern environments, cooler environments, or both) or September (in more southern and warmer environments).

Sources of nitrogen fertilizer

There are many different sources of N for plant growth, but the predominant fertilizer forms are urea ($CO(NH_2)_2$), ammonium nitrate (NH_4NO_3), ammonium sulphate ($(NH_4)_2SO_4$), monoammonium phosphate ($NH_4H_2PO_4$), diammonium phosphate ($(NH_4)_2HPO_4$), ammonium polyphosphate ($(NH_4PO_3)n$), calcium ammonium nitrate ($CaCO_3 + NH_4NO_3$), and urea ammonium nitrate (urea + ammonium nitrate).

For pasture, urea and ammonium nitrate have been the two main forms of N fertilizer used. Ammonium nitrate is not as prone to surface volatilization as urea. However, ammonium nitrate has recently been unavailable for farm use due to concerns for homeland security since it can be used to make explosives. Teutsch et al. (2005) compared the effect of different N sources on yield and quality of stockpiled fescue. Although, the source of N had no significant effect on CP, ADF, NDF and TDN, yield was influenced by it. They found that urea gave the lowest yield suggesting that N losses to the environment had likely occurred. Urea ammonium nitrate also gave low yield due to leaf burn and delayed growth. The yield response from broiler litter was similar to that from urea; broiler litter also consisting of organic forms of N likely didn’t mineralize completely in the first growing season. Ammonium nitrate and ammonium sulfate produced the highest yield as they were least likely to be volatilized. Sulfur from ammonium sulfate didn’t have significant effect on yield which as the authors suggested was because the soil of the experimental sites might have already been rich in sulfur. Urea is a low-cost source of N with high N content (46%), is easy to store and transport and certain precautions can be used to reduce surface volatilization such as treatment with urease inhibitors like Agrotain and incorporation in the soil after surface application. Efficiency of yield response to N depends not only on source and time of application but also on soil moisture. Yield response to N will be low in dry soils without rain, but even with small amounts of rain, tall fescue has good potential for fall growth.

Nitrogen cycling under pasture

Nitrogen is essential for life as it is the key constituent of amino acid, nucleic acid and ATP. It is the most abundant uncombined element on the earth which occupies about 78 % by volume (76% by weight) of the earth's atmosphere. Contrarily, N is also often the most limiting factor for biomass produced by living organisms in any natural ecosystem including pasture (Whitehead, 1995), as plants and animals cannot utilize gaseous N directly and has to be fixed. The various transformations of N in the atmosphere, the soil and the living organisms are collectively known as the N cycle (Whitehead, 1995). The range of transformations is wider for N relative to other nutrient elements such as phosphorus and potassium and it is partly due to the fact that N occurs in many different organic and inorganic compounds, in different state; some gaseous and some solid, and has a wide range of oxidation state from -3 (in ammonia) to +5 (in nitrates) (Whitehead, 1995).

Nitrogen fixation is the process by which N gets combined with other chemical elements, e.g. hydrogen or oxygen. A small amount of fixation occurs in the atmosphere during thunderstorms, some of it occurs biologically through microorganisms (biological N fixation) and industries also fix N to form chemical fertilizers using the Haber-Bosch process. Biological N fixation is done by a few genera of bacteria which produce nitrogenase enzyme that converts

N_2 to ammonia. Depending on whether bacteria involved have a symbiotic relationship with plants, biological N fixation can be divided into symbiotic and non-symbiotic N fixation. Symbiotic relationship is usually formed between bacteria such as Rhizobium and legumes where bacteria provide plants with ammonia in exchange for carbohydrates. In pastures of North America, important legume species are alfalfa, red clover and birdsfoot trefoil (Whitehead, 1995). A few non-legumes are also known to form symbiotic relationship with N-fixing bacteria. In case of non-symbiotic N fixation (e.g. by *Azotobacter*), ammonia fixed by bacteria is metabolized by the bacteria themselves.

Nitrogen mineralization is the process by which organic N is converted by soil micro-organisms to plant-available inorganic forms like ammonium (NH_4^+) and nitrate (NO_3^-). Change of organic N to NH_4^+ is called ammonification which is carried out by a wide range of heterotrophic micro-organisms, most of which are aerobic in nature. These microorganisms produce various enzymes like proteases and deaminases which hydrolyze specific substrates to produce NH_4^+. Similarly, change of NH_4^+ to NO_3^- is carried out mainly by two groups of autotrophic bacteria which are anaerobic in nature. Nitrogen mineralization is almost entirely a microbial function. The reverse of mineralization is immobilization. Immobilization is the assimilation of inorganic N by soil microorganisms. Immobilization may also occur from the reaction of amino acids and/or ammonia with phenolic and quinone-type compounds derived from the degradation of lignins. Both mineralization and immobilization occur continuously in soils and are closely related with each other through their dependence on the soil microbial population. The population of micro-organisms present in the soil derives its energy from the decomposition of organic matter which results in the continuous mineralization of C and N

(Windsor and Pollard, 1956). During decomposition, some of the C is assimilated and immobilized by the microbial biomass and some is mineralized into CO_2 due to microbial respiration which is lost into the environment. Unlike C, N is mineralized slowly and there may be a lag period of months or even years to be mineralized (Whitehead, 1995). Mineralized N is either taken by micro-organisms, plants, remains in the soil-plant system or is lost through leaching, ammonia volatilization and denitrification. Denitrification is a microbially facilitated process in which nitrate is reduced to produce molecular nitrogen (N_2). It takes place under anaerobic conditions.

Relationship between carbon and nitrogen mineralization

There is a close relationship between C and N in soil organic matter due to the microbial need and function. During the initial phase of decomposition, there is an increase in microbial biomass and C is used for energy and N for protein production. Only the mineralized N that is surplus to microbial requirement is released as net mineralized N and possibly used by plants. But if the decomposing organic material is low in N concentration, there will not be release of inorganic N to the soils for plant uptake. Whether N is mineralized or immobilized therefore hugely depends on the C:N ratio of the decomposing organic material. When C:N ratio is >30, soil micro-organisms are considered to consume N, causing N deficiency for the plant. This ratio decreases with evolution of CO_2 via decomposition and microbial demand for mineral N will decrease. When C:N ratio is $\leq$25, continuous mineralization is considered to occur. The C:N ratio of grass roots and leaf litter is usually between 40 and 60 when little or no fertilizer is applied resulting in net immobilization; but C:N ratio decreases to 25-30 for roots and <25 for litter, because of which decomposition induces slow mineralization (Whitehead, 1995). The microbial biomass has an average C:N ratio of between 5 and 8. After the initial phase of decomposition, microbial biomass usually declines which causes increase in net mineralization. The rate of turnover of N from dead microbial cells is about five times that of humified soil organic N (Stevenson and Cole, 1999). Also, soil fauna like protozoa and nematodes feed on soil micro-organisms, utilize some of the C and N for their own biomass and some of the C for respiration and excrete some of the N in soluble forms, which increases net mineralization of soil (Clarholm, 1985).

Carbon:N ratio is important in predicting net mineralization or immobilization but it can be misleading at the same time. For example, highly lignified organic materials decompose slowly releasing relatively low amount of C for microbial growth, because of which less N would be immobilized than suggested by their C:N ratio. The ratio of readily available C to readily available N appears to be more important (Whitehead, 1995).

Typical concentration of N in the top 15 cm of soil is usually 0.3-0.6% of soil dry weight under long-term pasture of temperate regions (Whitehead, 1995). Nitrogen gets into the soil of pastures through application of fertilizers and manures, through the deposition of excreta by grazing animals and a small amount through the deposition of ammonia and nitrate from the atmosphere. Nitrogen escapes the pasture through removal of grass or livestock products, and other losses including leaching, ammonia volatilization and denitrification. This in turn, can cause problems like eutrophication of terrestrial and aquatic ecosystems, water pollution and air pollution. The N requirement for optimum growth of plants supersedes the N required by animals for milk and meat production; hence, about 75 to 90% of N ingested by dairy cows is excreted in a grazing system (De Klein and Monaghan, 2011). Usually, N from the excreta of grazing animals is huge and can’t be utilized by plants leading to N loss. N is immediately available from urine as a result of hydrolysis of urea to NH_4^+ which is subsequently nitrified to NO_3^-, however, dung being largely in organic form is slow to mineralization.(De Klein and Monaghan, 2011); thus urine patches in pastures are primary sources of N loss.

Management practices to reduce N loss from pastoral systems as summarized by De Klein and Monaghan (2011) include: (i) lowering stocking rate and reducing N content of the feed which surely has to be considered within the boundary of profitability and productivity; (ii) applying fertilizer and manure only at the time when plants can take it; (iii) reducing grazing during wet seasons where chances of losses are maximum; and (iv) use of nitrification and urease inhibitors.

Carbon and nitrogen sequestration under pastures

Soils are the largest terrestrial organic C pools in the biosphere, storing more C than the plants and the atmosphere combined (Schlesinger, 1997), therefore, recent emphasis has been placed on the importance and need of soil to sequester C from atmospheric CO_2 into soil organic matter as a mitigation strategy for greenhouse gas emissions and global warming (Swift, 2001). The role of soil organic carbon (SOC) as a key control factor in soil fertility and agricultural production is long known (Jobbágy and Jackson, 2000).

Several factors can affect SOC storage in agricultural soils including climate, crop regime, soil type and management. In relation to the crop regime level, factors such as the production of plant and root biomass, depth of rooting and type of root systems can influence SOC storage, especially in the subsurface soil layers (Carter and Gregorich, 2010). As summarized by Jobbágy and Jackson (2000) from diverse arrays of soils and vegetation types, regional patterns of SOC are positively associated with clay content because of the stabilizing effect of clay on SOC, also positively associated with mean annual precipitation as it constraints both plant production and decomposition with greater response on production but negatively associated with mean annual temperature since in higher temperature, the rate of decomposition is higher than that of production.

Perennial grasses influence SOC stocks within the 0–1 m soil depth, with most changes occurring in the surface 40 cm (Carter et al., 1997). This is because 70-90% of the roots of tall fescue or other perennial grasses are found at 0-30 cm soil depth (Bolinder et al., 2002). Tall fescue has half of its root biomass at 0-20 cm soil depth (Nie et al., 2008). However, some deep rooted grasses have the ability to sequester SOC deep in the soil (Fisher et al., 1994). Glover et al. (2010) showed that perennial grass fields maintained more root C, more SOC and more total soluble N (TSN) than annual crop fields. Guo and Gifford (2002) observed that conversion of crop land to pasture caused substantial C accumulation below 1-m depth.

Temperate pastures in the northeast USA are highly productive and have potential as significant C sinks (Skinner, 2008). The pastures in southeastern USA are also rich in C and N, but there is limited information on the rates of SOC and TN sequestration in managed pastures (Franzluebbers, 2010) and under different forage systems, management conditions and environments (Franzluebbers et al., 2012). An eight year study of tall fescue pasture in the southern Piedmont region found that SOC and TN sequestration rates were greater under various grazed management regimes than under hayed management (Franzluebbers et al., 2012). This study was based on samples taken from different depths within the surface 20 cm. The rate of SOC and TN sequestration under grazed management regime was 1.54 ± 0.10 Mg C ha^{-1} yr^{-1} and 0.129 ± 0.006 Mg N ha^{-1} yr^{-1} respectively, and under hayed management was 0.082 Mg N ha^{-1} yr^{-1}.

Tall fescue with deep-rooting ability (Nie et al., 2008 ; Franzluebbers and Stuedemann, 2009), can help sequester C throughout the soil profile. Carter and Gregorich (2010) in their study of a seven-year old tall fescue field observed a significant increase in the stock of SOC and TN in the 0-60 cm soil depth as compared to the SOC and TN levels established over 15 years of small grain production. Much of the SOC and TN gain were concentrated in the 0-10 cm soil depth but significant increase in the 40-60 cm soil depth supported the ability of tall fescue to increase SOC stocks deeper. A correction for bulk density caused by soil consolidation and vehicular trampling in the 10-40 cm soil depth was done as bulk density can limit root growth in soil.

Endophyte-infected vs endophyte-free in case of carbon sequestration

Endophyte infected (E+) tall fescue has been associated with greater stocks of SOC than tall fescue with no (E-) or low levels of endophyte infections. Franzluebbers et al. (1999) studied pastures in Georgia and found that the difference in SOC storage in the 0 to 30 cm depth was 1.8 ± 2.0 Mg ha^{-1} (mean ± standard deviation among experimental sets) greater under high than low endophyte infection. The difference in SOC was thought to be mainly from E+ tall fescue litter decomposing slower than E- tall fescue litter (Franzluebbers and Stuedemann, 2005; Siegrist et al., 2010). Endophyte-produced metabolites such as alkaloids and phenolics are considered as primary agents for slowing decomposition rates of fescue litter (Siegrist et al., 2010); directly inhibiting decomposition by being toxic to the microbial community (Elmi et al., 2000; Rudgers and Clay, 2007) and indirectly by deterring insect herbivory and changing microbial species composition (Franzluebbers et al., 1999; Rudgers and Clay, 2007). An outdoor microcosm study in Argentina found that E+ ryegrass leaf litter decomposed slower than E- leaf litter suggesting that endophyte-produced metabolites deter decomposition. Siegrist et al. (2010), however, suggested that the direct effect of endophyte on litter decomposition was small despite greater alkaloid concentration. They questioned whether alkaloid could be the primary mechanism driving the direct effect. Soil microbial biomass C was not much affected by the endophyte-infection level but there were some discrepancies with the data. Franzluebbers et al. (1999) have proposed an interesting hypothesis that perhaps soil microbial biomass adapts gradually to accumulation of endophyte-infected tall fescue residues although initially inhibited by various endophyte metabolites which could be the reason of the discrepancy. Basal soil respiration was greater under low than high-endophyte infected fescue stands at 0-25 cm depth. Because of greater basal soil respiration and lower SOC under low endophyte infected tall fescue, Franzluebbers et al. (1999) concluded that high endophyte infection decreases the quality of organic substrate, allowing more SOC to accumulate.

Franzluebbers and Hill (2005) found that for short-term exposure (32 days) to endophyte infection with addition of E+ leaves to soil reduced C mineralization and SMBC as compared to E- leaf addition, but both were greater than in the unamended soil. Contrarily, net N mineralization and soil microbial biomass N were higher under E+ exposed soils when exposed to short term infection. For long-term history (10 years) of endophyte infection, cumulative C mineralization was not different for E+ and E- but per unit of SOC was greater for E- than E+. Net N mineralization and SMBC were not affected by long term exposure.

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CHAPTER 2

FALL STOCKPILED TALL FESCUE RESPONSE TO NITROGEN FERTILIZER DEPENDS ON SOIL BIOLOGICAL ACTIVITY

ABSTRACT

Nitrogen (N) fertilizer application to tall fescue during late summer is common to promote herbage accumulation prior to winter grazing. However, little is known of the N supplying capacity of soil under tall fescue pastures. We hypothesized that pastures with low reservoir of biologically active soil N would respond significantly to late summer N fertilizer application, while pastures with high reservoir would not respond. Pastures with a diversity of management (22 in 2015 and 35 in 2016) were tested in North Carolina, Virginia, West Virginia, and Georgia. Each field had four N treatments (0, 45, 90, 135 kg N ha^{-1}) replicated four times in a randomized complete block design (16 plots, 3×6 m each). Urea fertilizer was applied at the beginning of September 2015 and 2016. Forage was allowed to grow until harsh winter conditions set in (December to January), at which time plots were mechanically harvested to determine dry matter (DM) and crude protein (CP) production. Harvest height was $\geq$10 cm in 2015 and $\geq$10 cm and 5-10 cm in 2016. Soil was sampled at 0-10 cm depth in August/September of each year. Soil N supplying capacity was assessed with inorganic N accumulation during aerobic incubation at 25^{0}C for 24 days (i.e., net N mineralization). Other soil biological assessments were soil microbial biomass C (SMBC), particulate organic C and N, cumulative C mineralization, and the flush of CO_2. Forage samples were scanned with near infra-red spectroscopy (NIRS) to determine CP. Relative yield response for $\geq$10 cm harvest height at threshold yield efficiency of 5 kg DM kg^{-1} N was highly negatively correlated to the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC (r^2 = 0.86, 0.76, 0.80, and 0.65 respectively). Similarly, actual yield response to N fertilizer was highly negatively correlated to the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC ($r^2 = 0.59$, 0.53, 0.56, and 0.46 respectively). Economically optimum N rate (EONR) was also highly negatively correlated to the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC. The relationships between relative yield response and EONR with the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC were also significant at a greater threshold yield efficiency of 10 kg DM kg^{-1} N and at harvest height of 5-10 cm. For harvest height of 5-10 cm, significance of correlation was lower than that for $\geq$10 cm, suggesting a less responsive forage layer to N fertilization. Total N and C had weak correlation with relative yield response at threshold yield efficiency of 5 kg DM kg^{-1} N ($r^2 = 0.34$, and 0.45 respectively), and also with actual yield response and EONR at either threshold efficiency. Particulate organic N and C were not correlated to yield responses. Crude protein and field moisture of harvested forage correlated positively with N rate applied. Forage $\geq$10 cm had greater CP and field moisture than that cut at 5-10 cm height. Sites with greatest yield response to N fertilizer had lowest soil biological quality measures. Our hypothesis was accepted – fall-stockpiled yield response to N fertilizer declined with increasingly greater biologically active soil N. Adjusting N fertilizer recommendations for fall-stockpiled tall fescue based on soil biological activity should be pursued further.

INTRODUCTION

Nitrogen (N) recommendations for stockpiled tall fescue in the USA are not based on soil testing. Potential soil tests for N availability are either too time consuming or insufficiently accurate. Soil biological activity evaluations are generally not considered for soil testing by most laboratories. Nitrogen recommendation for tall fescue pasture management for hay or silage in North Carolina is 112 to 224 kg ha^{-1} yr^{-1}, and rates can be reduced by 25% for continuous grazing and by 50% for rotational grazing. (Castillo et al., 2016). A greater rate is suggested for sites with low fertility index (described as a site where yield increase is expected with the recommended N fertilizer rate) but this recommendation doesn't mention soil biological quality. In Virginia, the recommended N rate is 70-90 kg ha^{-1} (Johnson and Smith, 2004), independent of management. Soil testing is only used to recommend level of phosphorus, potassium and lime. Nitrogen recommendation for stockpiled tall fescue in Tennessee is 70 kg ha^{-1}(anonymous, (n.d.)), independent of soil quality or condition. Similarly, N recommendation in Georgia and Mississippi is in the range of 45-70 kg ha^{-1} (Hancock and Josey, 2008; Lemus, 2008) without soil testing. The only soil testing for N in the southeastern USA is for inorganic N by some laboratories.

A hypothesis was developed that soil N supplying capacity would affect yield response to N fertilization in stockpiled tall fescue pastures, such that sites with high biologically active soil N would have little to no yield response and sites with low biologically active soil N would have high yield response to N fertilizer.

MATERIALS AND METHODS

Field set up and design

Experimental sites were established on pastures containing a dominance of tall fescue on several farms and research stations, mostly in North Carolina and Virginia, but also a few in West Virginia and Georgia (Appendix C; Fig C.1, C.2, C.3). A total of 22 fields were selected in 2015 and 35 fields in 2016. Management of pastures included rotationally grazed, occasionally grazed, and mowed/hayed, the effect of which was expected to alter soil biological activity. Also, pastures with both wild-type and novel endophyte were chosen to represent typical producers in the southeastern USA. Detailed information of each site is listed in Table 1.1.

Each field had a total of 16 plots (3×6 m each) composed of four replications of four N fertilizer treatments (0, 45, 90 and 135 kg ha^{-1}) in a randomized complete block design. For both years, stockpiling was started in August and N applied in the form of urea in early September. Uncoated urea was used for most sites, but urea coated with urease inhibitor was used at the Georgia locations in 2016. During the period of stockpiling, each field was protected against grazing animals.

Soil sampling

Soil was sampled at 0-10 cm depth from each of four replicates within each field using a probe of 4-cm diameter. Eight cores were composited per replicate sample for a total volume of 1005 cm^3. Samples were collected in August or September prior to fertilization.

Soil processing

Soil samples collected from a total of 57 field sites (n=228) were oven dried (55˚C, 3 d) and sieved to pass 4.75 mm sieve by gently crushing with a pestle. Stones and large pieces of organic detritus were removed. Sieve opening of 4.75 mm was a reasonable compromise to achieve balance between obtaining a homogenous sample and keeping soil aggregates partially intact (Franzluebbers, 1999). Aggregates are important as they help in maintaining stability and aeration during incubation, an important aspect for routinely characterizing soil biological activity (Franzluebbers, 2016). Sieved soil samples were thoroughly mixed in sampling bags before taking subsamples.

Soil bulk density was calculated from the mass of oven-dried soil divided by the soil core volume. Mathematically,

$$\text{Soil bulk density} = \frac{Oven\ dried\ soil\ wt.(g)}{soil\ core\ volume\ (cm\hat{\ }3)}$$

Soil porosity was calculated as:

$$\text{Soil porosity} = 1- \frac{bulk\ density}{particle\ density};$$

assuming particle density = 2.65 Mg m^{-3}

Laboratory incubation

Laboratory incubation was used to determine C and N mineralization and SMBC following an established protocol (Franzluebbers and Stuedemann, 2007). For each soil sample, two subsamples of 50 ± 0.01 g were weighed into 60-mL graduated glass bottles and volume recorded following light adjustment to settle. Upon calculating soil porosity, water was added to attain 50% water filled pore space (WFPS), which was calculated as:

$$H_2O \text{ need to achieve 50\% WFPS (mL)} = 0.5 \times \text{porosity} \times \text{soil volume (mL)}$$

Duplicate subsamples were placed in a 1-L canning jar along with a 30-mL Nalgene bottle containing 10 mL of 1 *M* NaOH to trap evolved CO_2 and a vial containing 10 mL of water to maintain humidity. The reaction assessed in this setup was:

$$2NaOH_{(aq)} + CO_{2\,(g)} \rightarrow Na_2CO_{3\,(aq)} + H_2O_{(l)}$$

Carbon mineralization and the flush of CO_2

Soil samples were incubated in a constant temperature cabinet at 25 °C for up to 24 days. At 3 and 10 days of incubation, NaOH traps were removed, capped and later titrated with known concentration of ~1M HCl. Replacement of NaOH traps took a few seconds. For titration, each trap was worked on individually and caps removed in sequence. After removing caps, sufficient 1.5 M $BaCl_2$ was added to the NaOH traps to precipitate bicarbonate as $BaCO_3$. The reaction was:

$$Na_2CO_{3\,(aq)} + BaCl_{2(aq)} \rightarrow BaCO_{3(s)} + 2\,NaCl_{\,(l)}$$

Few drops of phenolphthalein color indicator and a small magnetic stir bar were also added to NaOH traps. Each trap was then placed on a magnetic stir plate and HCl was slowly added until the color changed from pink to colorless (pH~9.3). A blank NaOH trap sequenced after every 11th sample was incubated in the same manner, but without exposure to soil and titrated in similar manner. HCl neutralized the remaining NaOH which did not form Na_2CO_3.

Quantity of CO_2 evolved from each soil sample was calculated as (Franzluebbers, 2016):

$$CO_2\text{-C (mg kg}^{-1}\text{ soil)} = \text{(mL [blank] – mL [sample]) x N x M / S}$$

where, N = normality of acid (mol L^{-1}), M = mass conversion from cmol C to g C (6000), and S = soil weight (g)

The value obtained from above equation at 0-3 days of incubation is referred to as the flush of CO_2. Cumulative carbon mineralization was calculated at 0-24 days of incubation. First 0-3 day CO_2-C value was calculated, then the NaOH traps were replaced with fresh NaOH and again incubation was carried out under similar conditions for 3-10 days at which point the NaOH traps were removed and titrated. At day 10, the NaOH traps were replaced with fresh NaOH and incubated for 10-24 days. The CO_2-C values from 0-3 days, 3-10 days and 10-24 days were added to get a cumulative CO_2-C value for 24 days. Also, on Day 10 of incubation, one of the 60 mL jars of soil was removed from the canning jar and fumigated with chloroform to determine SMBC. Incubation for C mineralization was calculated with only one 60 mL jar (50g soil) for 10-24 days.

Soil microbial biomass carbon (SMBC)

Soil microbial biomass C was determined using the chloroform-fumigation incubation method (Jenkinson and Powlson, 1976). On Day 10 of incubation, one of the 60 mL jars of soil was removed from canning jars and placed in vacuum desiccator (about 24 samples in one dessicator). A 50 mL beaker with boiling chips and 30 mL of chloroform was placed into each desiccator. Desiccators were closed and then individually vacuumed for about 30 seconds following boiling of chloroform. Desiccators were paced in the dark at room temperature for 18-24 hours. The next day (Day 11), desiccators were opened, chloroform removed and vapors removed with repeated vacuum. Soil jars were taken out from desiccators and put into fresh canning jars along with 10 mL of NaOH and 10 mL of water. Soil was incubated at 25˚C from 11-21 days at which point NaOH traps were removed and titrated in similar manner and SMBC was calculated. An efficiency factor of 0.41 was used (Voroney and Paul, 1984):

$$\text{SMBC (mg kg}^{-1}\text{ soil)} = (\text{mL [blank]} - \text{mL [sample]}) \times \text{N HCl} \times 6 \times 1000 / ((\text{g soil}) / k_C)$$

where, 6 = equivalent weight of C and $k_C = 0.41$ (efficiency factor)

Net nitrogen mineralization and available nitrogen

Net N mineralization was calculated as the difference in inorganic N (NH_4^+ and NO_3^-) between Day 0 and Day 24 of incubation. For calculating inorganic N at Day 0, 10 g of oven dried, sieved and ball milled soil samples were weighed into 30 mL Nalgene bottles. 20 mL of 2 M KCl was added to each bottle and shaken for 30 minutes in a reciprocating shaker. Two blanks containing only 20 mL of KCl and one standard containing 5 g of standard soil sample and 20 mL of KCl were also paced every 50-60 soil samples. KCl extracts were filtered using Whatman no. 5 paper. Extracts were then analyzed for NH_4 and NO_3 + NO_2 using Bran Luebbe Auto-Analyzer 3 using salicylate nitroprusside and hydrazine methods, repectively. The process was repeated at Day 24 using oven-dried subsample (55˚C, 3 d) after sieving through 2 mm sieve screen. Available N was calculated as summation of initial inorganic N and net N mineralization.

Particulate organic matter and texture analysis

Previously fumigated 50 g subsamples were oven-dried (55˚C, 3 d) and transferred into 125-mL Nalgene bottles. 100 mL of 0.1 M sodium pyrophosphate was added to each bottle. A blank containing only 100 mL of 0.1 M sodium pyrophosphate was used every batch. The bottles were then shaken in a reciprocating shaker for 12-16 hours. Soil solution was transferred into 1-L cylinders and the final solution volume was brought to 1 L with de-ionized water. Every cylinder was mixed 10 times using a mechanical stirrer and allowed to settle untouched thereafter for 5 hours. At 5 hours, the level of a hydrometer was recorded along with mean room temperature during the period of hydrometer reading. Clay concentration was related to the density of solution after larger particles settled to the bottom of the cylinder according to the following equation (Gee and Bauder, 1986):

Clay (g/g) = ((((°C - 20) *0.36) + hydrometer reading (mg/L)) - blank) / soil weight (g)

The soil solution was then transferred to a screen with 0.053 mm openings and washed with de-ionized water until only sand and particulate organic matter remained. These sand-sized particles were transferred to a jar and then oven-dried (55°C, 3 d). Sand was calculated as:

$$\text{Sand concentration (g/g soil)} = \frac{Wt.of\ sand\ obtained\ from\ sieving\ and\ drying}{Weight\ of\ soil}$$

Silt concentration was calculated as the difference between unity and summation of clay and sand content. The dried sand-sized fraction was ball milled to homogenize the sample and particulate organic matter C and N fractions were determined by dry combustion using Leco TruMac CN analyzer.

Soil testing

Oven dried and sieved (4.75mm) subsamples of about 100 g soil were sent to the North Carolina Department of Agriculture and Consumer Services (NCDA & CS) state soil-testing lab for macro and micronutrient analysis. Soils were characterized for humic matter (HM), cation exchange capacity (CEC), pH, base saturation, phosphorus, potassium, calcium, magnesium, sulfur, manganese, zinc, copper and sodium concentration using Mehlich-3 extraction method (Mehlich, 1984).

Forage harvesting and dry matter analysis

Forage was harvested in December/January of both years. Each plot was trimmed around its border before harvest. For the first year of research, forage was harvested at 10 cm height from 10 m^2 area in each of 16 plots. For second year, forage was harvested at two different heights; (i) $\geq$10 cm and (ii) 5-10 cm from the ground surface. The objective of taking two different heights for second year of research was to compare and contrast DM production and nutritive value between two different forage heights. Total wet weight was determined in the field. Subsamples were taken, weighed wet and weighed again after oven-drying to constant weight (55˚C, $\geq$ 3 d) to determine DM production.

Forage nutritive value evaluation

Harvested tall fescue forage subsamples were oven dried (55˚C, $\geq$ 3 d), ground to pass a 1 mm screen in a Wiley mill, thoroughly mixed, and a further subsample subjected to evaluation for N and C concentration using near infrared spectroscopy (NIRS). Samples were scanned using a model 5000 NIRS with WinISI version 1.5 software (Foss North America, Inc., Eden Prairie, MN). Calibration was done by evaluating spectra for outliers (‘H’ > 3.0) prior to sample selection. The ‘H’ statistic (0.6) was used to select samples with different spectra. Total samples selected with different spectra was 90 out of a possible 320 (20 sites x 16 plots/site; 2 sites were not harvested because of livestock intrusion) in 2015 and 189 out of a possible 1120 (35 sites x 16 plots/site x 2 heights) in 2016. Selected samples were subsequently analyzed for N concentration using a Leco TruMac CN combustion analyzer. Calibration equations were developed for N using modified PLS regression with four cross validations (Table 2.2). The equation was then applied to all samples to estimate N concentrations.

Crude protein of forages was determined by multiplying total N by 6.25 due to the fact that protein is 16% N (Henneberg, 1865; Rubner, 1885).

Statistical Analysis

Threshold yield efficiency (TYE)

Forage DM estimates were adjusted to 15% moisture content as industry standard for hay production. Stockpiled tall fescue DM was regressed upon N fertilizer rate. Regression on rates of 0 to 135 kg ha^{-1} used an exponential rise to maximum equation function (single, 3 parameters) in SigmaPlot version 12.5.

Mathematically,

$$f = Y_0+a*(1-\exp(-b*x))$$

where, f is the yield at a particular level of x (N fertilizer rate), Y_0 is the Y-intercept without N fertilizer, a is the maximum yield response to additional N, and b is the exponential rise coefficient. Only if the response didn’t fit an exponential rise to maximum equation, linear equation was used instead. Two different TYE were calculated to determine relative yield response and economically optimum N rate to cover the fluctuations in price of both hay and N fertilizer. Threshold yield efficiencies of (a) 5 kg DM kg^{-1} N (will be referred as minimum) and (b) 10 kg DM kg^{-1} N (will be referred as maximum) were considered based on example price ratio of \$1 kg^{-1} N fertilizer and \$0.20 kg^{-1} hay and \$2 per kg^{-1} N fertilizer and \$0.20 kg^{-1}

hay, respectively. Current hay prices might be considerably lower (\$0.08 kg^{-1} hay or \$73 short ton^{-1}; Matt Poore, personal communication), and the implication of this would be for TYE of 12.5 and 25 DM kg^{-1} N at fertilizer costs of \$1 and 2 kg^{-1} N, respectively. It was felt that the lower TYE values would provide fairer evaluation of the need for N fertilizer in this study.

Yield response parameters

Four yield response parameters of stockpiled tall fescue to N fertilization were calculated: (i) actual yield response (iii) N uptake response (iii) relative yield response and, (iii) economically optimum N rate. Both non-linear and linear regression analysis of yield response to N fertilization was done in SAS version 9.4 (SAS Institute, Cary, NC) using PROC NLIN and PROC GLM respectively. For non-linear regression analysis, exponential decay function was used (single, 3 parameters).

Mathematically,

$$f= Y_{0} + a*\exp(-b*x)$$

where, f is the response at a particular level of x (soil biological activity), Y_0 is a basal response that is independent of soil biological activity, a is the maximum response, and b is the exponential decay coefficient. Only if non-linear regression did not fit the data, linear regression of yield on N rate was determined.

PROC MEANS was used to find the average among the replications. t test ‘least significant difference’ was used to make multiple comparisons between dry matter, crude protein and moisture content of harvest forage for individual sites.

PROC REG followed by stepwise selection was used for evaluating the effect of temperature and precipitation on yield response of stockpiled tall fescue to N fertilization.

Crude protein and field moisture content of harvested tall fescue forages were analyzed across 57 sites of study. Exponential rise to maximum equation function in SigmaPlot version 12.5 was used to see the relationship between average CP and N rate and similarly between average field moisture and N rate. The significance test was done by PROC NLIN in SAS version 9.4 (SAS Institute, Cary, NC).

Actual yield response

Actual yield response of tall fescue to N fertilization was calculated as the difference between yield at 135 kg N ha^{-1} and yield without N fertilizer from regression function. Nitrogen rate of 135 kg ha^{-1} reflected the highest yield with applied N fertilizer.

Nitrogen uptake response

Nitrogen uptake response was calculated as the difference between N uptake at 135 kg N ha^{-1} and N uptake at 0 kg N ha^{-1} from regression function. Nitrogen rate of 135 kg ha^{-1} reflected the highest N uptake with applied N fertilizer.

Relative yield response

Relative yield response to optimum N was calculated as 100 × (yield at optimum N rate – yield at 0 N rate)/yield at 0 N rate. Both minimum TYE of 5 kg DM kg^{-1} N and maximum TYE of 10 kg DM kg^{-1} N were used to determine the optimum N rate. Relative yield response considered both agronomic and economical response of tall fescue to N fertilizer, whereas actual yield response considered only the agronomic response of N of tall fescue to highest applied N rate regardless of the cost of hay and N fertilizer.

Economically optimum N rate (EONR)

Economically optimum N rate was calculated at both TYEs of 5 and 10 kg DM kg^{-1} N. Economically optimum N rate was not allowed to exceed the highest N application rate of 135 kg ha^{-1}.

Weather data

Data on daily average maximum temperature, average minimum temperature and rainfall were collected from nearest weather stations using CRONOS (Climate Retrieval and Observations Network of the Southeast) database. Precipitation data for the duration of September-November in 2015 and 2016 were compared with the 30-year average weather data (1961-1990 normals). The ratio of actual to 30-year normal precipitation was taken as the index of weather condition. If the ratio was $\leq$0.75, then the condition was below normal precipitation.

If the ratio was ≥ 2, then the condition was above normal precipitation. Near normal precipitation was with ratio >0.75 and <2. In 2015, seven fields had above normal precipitation, two fields had below normal precipitation, and two fields were not harvested because of livestock intrusion. In 2016, four fields had above normal precipitation and 14 fields had below normal precipitation. Due to adverse weather conditions, only 28 out of 57 field sites had near normal conditions.

RESULTS

Soil biological activity and yield response of stockpiled tall fescue

Soil biological activity across 57 field sites ranged from 90 to 723 mg flush of CO_2-C kg^{-1} soil, 433 to 2116 mg kg^{-1} soil of cumulative C mineralization over 24 days, 508 to 2798 mg SMBC kg^{-1} soil, 34 to 416 mg kg^{-1} soil of net N mineralization over 24 days, 0.03 to 0.63 mg g^{-1} soil of particulate organic N, and 0.6 to 9.0 mg g^{-1} soil of particulate organic C. Field sites with relatively short history of pasture establishment had lower values of soil biological activities than that of old pastures.

Actual yield response of stockpiled tall fescue to N fertilization ranged from 0 to 1238 kg ha^{-1} across 55 harvested sites at $\geq$10 cm and ranged from 8 to 430 kg ha^{-1} across 35 harvested sites at 5-10 cm (Table 2.4 and 2.5). Nitrogen uptake response across 55 sites ranged from 0 to 25 kg ha-1 at $\geq$10 cm and from 0 to 19 kg ha-1 at 5-10 cm. At $\geq$10 cm harvest height, 45 of the 55 sites were non responsive (<25% relative yield response), four sites were somewhat responsive (relative yield response between > 25% and < 50%) and six sites were responsive ($\geq$ 50% relative yield response) at threshold yield efficiency of 5 kg

DM kg^{-1} N. At threshold yield efficiency of 10 kg DM kg^{-1} N and $\geq$10 cm harvest height, 51 sites were non responsive, three field sites were somewhat responsive and only one site was responsive. At 5-10 cm, all the 35 sites were non-responsive at both threshold yield efficiencies of 5 and 10 kg DM kg^{-1} N.

Temperature and precipitation effects on yield response to N fertilization

Weather conditions could be an important factor influencing the yield and yield response of tall fescue to N fertilization. Cumulative rainfall, average maximum temperature and average minimum temperature from September to November were analyzed against the yield response parameters in SAS version 9.4 (SAS Institute, Cary, NC) using PROC REG and stepwise selection. Average maximum temperature and average minimum temperature were not significant and thus, were not selected as variables for yield response to N fertilization. Precipitation however, was a significant variable and thus, efforts were made to classify the fields into above, near and below normal precipitation categories.

I. Near normal precipitation

Relative yield response as affected by soil biological activity ($\geq$10 cm harvest height)

Field sites which had actual to normal precipitation ratio >0.75 and <2 were categorized under near normal precipitation. Of the total 57 sites, 28 sites had near normal precipitation. Relative yield response of stockpiled tall fescue at threshold yield efficiency of 5 kg DM kg^{-1} N ranged from 0 to 175% (Table 2.4 and 2.5). Twelve out of the 28 harvested sites didn't respond to N fertilizer while other fields had responses to N fertilizer ranging from as small as 1 % (Site 15-21 in Clay County NC) to 175% (Site 15-08 in Durham County NC). Site 15-21 was a rotationally grazed >40-year pasture and site 15-08 was a newly established 1-year old pasture. Of the 28 field sites, 22 had $\leq$25% response, 3 had between 25 and 50% response, and 3 had $\geq$50% relative yield response.

Both total and labile C and N fractions of soil organic matter were regressed against relative yield response to assess the influence of a soil biological gradient on potential yield response. Non-linear regression analyses of relative yield response at threshold yield efficiency of 5 kg DM kg^{-1} N versus the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC were highly significant (r^2 = 0.86, 0.76, 0.80, and 0.65, respectively; Table 2.7 and Fig. 2.1). Non-linear regression analyses of relative yield response at threshold yield efficiency of 5 kg DM kg^{-1} N versus total C and total N were also significant, but weaker (r^2 = 0.45 and 0.34 respectively; Table 2.7 and Fig. 2.3). Partially decomposed particulate organic C and N were not significantly related to relative yield response of stockpiled tall fescue to N fertilization at 5 kg DM kg^{-1} N (Table 2.7). These responses were assessed on mean values at each site, and similar relative response occurred for individual replicates for each site (Figure 2.19). Mean values of each site were subsequently chosen for other analyses, because the large-scale variation among sites was more important to assess than the fine-scale replicate variation that included greater contribution from random sampling variations of small plots.

The relationship between soil biological quality measures and relative yield response at a greater threshold yield efficiency of 10 kg DM kg^{-1} N was also tested. At this higher threshold yield efficiency, fewer sites responded to N fertilizer. Twenty out of 28 harvested sites didn't respond to N fertilizer and the greatest response of stockpiled tall fescue to N fertilizer was 84% (Site 15-08, Durham County, NC), which was less than half the magnitude at 5 kg DM kg^{-1} N (Table 2.5 and 2.6). Even at higher threshold yield efficiency, the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC were significantly correlated with relative yield response (r^2 = 0.86, 0.73, 0.81, and 0.59 respectively; Table 2.7; Fig. 2.2 and 2.4). Particulate organic C and N fraction were not significantly related to the relative yield response of stockpiled tall fescue to N fertilization at 10 kg DM kg^{-1} N (Table 2.7), and total C and N were weakly related (r^2 = 0.42 and 0.30, respectively).

The results support the hypothesis that soil biological activity alters yield response of stockpiled tall fescue to N fertilization. Sites with greater than 300 mg flush of CO_2-C kg^{-1} soil didn't respond to N fertilization.

Some measured physical and chemical properties were significantly correlated with relative yield response. The relationship between humic matter and relative yield response at threshold yield efficiencies of 5 and 10 kg DM kg^{-1} N was weak but significant (at a probability level of <0.05) (Table 2.8, Fig. 2.12). Similarly, cation exchange capacity, potassium and calcium levels in the soils explained $\leq$16% of the variation. Inorganic N was not significantly related with the relative yield response (Table 2.7). Available N was significantly related to relative yield response at both threshold yield efficiencies of 5 and 10 kg DM kg^{-1} N (r^2 = 0.75 and 0.76 respectively; Table 2.7, Fig. 2.3 and 2.4). Strength of this relationship was therefore more due to net N mineralization than inorganic N. Tall fescue yield was clearly dependent on mineralization of organic N.

Actual yield response as affected by soil biological activity ($\geq$10 cm harvest height)

The total range of actual yield response of stockpiled tall fescue to N fertilizer was 0 to 1238 kg ha^{-1} (Table 2.2 and 2.3). The association between the flush of CO_2 and actual yield response was explained by an exponential decay function ($r^2 = 0.59$; Table 2.7 and Fig. 2.5.). Similarly, non-linear regression analyses of actual yield response versus cumulative C mineralization, net N mineralization and SMBC were significant ($r^2 = 0.53$, 0.56 and 0.46 respectively; Table 2.7 and Fig. 2.5). Total N and C were also negatively correlated with actual yield response. Particulate organic N and C were not correlated with stockpiled tall fescue yield increase with N (Table 2.7).

Nitrogen uptake response as affected by soil biological quality ($\geq$10 cm harvest height)

Nitrogen uptake response of stockpiled tall fescue to N fertilizer ranged from 3 to 25 kg ha^{-1} (Table 2.4 and 2.5). Flush of CO_2 was significantly correlated to N uptake response ($r^2 = 0.25$, Figure 2.11). Cumulative C mineralization, SMBC, and net N mineralization were also significantly correlated to N uptake response ($r^2 = 0.24$, 0.36, and 0.37 respectively). Particulate organic C and N fractions were not correlated with N uptake response. Total C and N fractions were correlated with N uptake response ($r^2 = 0.36$ and 0.35 respectively).

Economically optimum N rate as affected by soil biological quality measures ($\geq$10 cm harvest height)

The range of EONR for stockpiled tall fescue at threshold yield efficiency of 5 kg DM kg^{-1} N was from 0 to 115 kg N ha^{-1} (Table 2.5 and 2.6) with a mean value of 17 kg ha^{-1} N. Thirteen sites didn't need any N fertilization. Non-linear regression analyses of EONR versus the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC were significant (r^2 = 0.63, 0.53, 0.60 and 0.53 respectively; Table 2.4 and Fig. 2.7). Particulate organic N and C were not correlated with EONR at 5 kg DM kg^{-1} N. Total N and total C were also significantly correlated with EONR at 5 kg DM kg^{-1} N (r^2 = 0.37 and 0.42 respectively, Table 2.7 and Fig.2.9).

At a higher threshold yield efficiency of 10 kg DM kg^{-1} N, EONR ranged from 0 to 29 kg ha^{-1} (Table 2.5 and 2.6). Non-linear analyses of EONR at 10 kg DM kg^{-1} N versus flush of CO_2, cumulative C mineralization, net N mineralization and SMBC were significant (Table 2.4 and Fig. 2.8).

Some physical and chemical properties of the soil were also tested against EONR. Humic matter, cation exchange capacity, potassium and calcium levels in the soil explained some percent of the variation in the relationship between EONR and soil biological quality. Inorganic N was not significantly correlated to EONR at 5 kg DM kg^{-1} N but was weakly correlated with EONR at 10 kg DM kg^{-1} N (Table 2.7). Available N was strongly correlated with EONR (Table 2.7).

Tall fescue yield response at 5-10 cm and $\geq$5 cm as affected by soil biological quality

Stockpiled tall fescue at 5-10 cm showed smaller relative yield response to N fertilizer at threshold yield efficiency of 5 kg DM kg^{-1} N as affected by the flush of CO_2 than that of $\geq$10 cm (Fig. 2.13). Similar were the cases with relative yield response at threshold yield efficiency of 10 kg DM kg^{-1} N, actual yield response (figure 2.14) and EONR at both threshold yield efficiencies as affected by cumulative C mineralization, net N mineralization, SMBC, total C and N (figure not shown). Like that for the $\geq$10 cm harvest height, particulate organic C and N were not significantly correlated to yield response parameters.

Relative yield response of tall fescue at $\geq$5 cm (summation of $\geq$10 and 5-10 cm harvest heights) had weaker association with the flush of CO_2 (Fig. 1.12) as compared with that at $\geq$10 cm.

Identifying the key variables to predict yield response to nitrogen fertilization

Soil biological activity measures along with total N and C were subjected to stepwise selection following PROC REG in SAS version 9.4 (SAS Institute, Cary, NC) to identify the most important variables to predict yield response of stockpiled tall fescue to N fertilization. The flush of CO_2 and SMBC were selected as key variables for actual yield response, N uptake response and EONR at threshold yield efficiency of 5 kg DM kg^{-1} N. Only the flush of CO_2 was selected as a key variable to predict relative yield response at both threshold yield efficiencies and EONR at 10 kg DM kg^{-1} N.

A principal component analysis was done using the flush of CO_2 and SMBC as they might have co-varied with each other. The first principal component explained 88% of the variation and the second principal component explained 12% of the variation. Since the value of eigenvectors for the flush of CO_2 and SMBC were similar (Table 2.3), both the variables were equally important in predicting actual yield response and EONR at 5 kg DM kg^{-1} N. Overall, the flush of CO_2 was a key variable to predict yield response of stockpiled tall fescue to N fertilization.

II. Below normal precipitation

Of the total of 57 field sites, 17 had below normal precipitation. At harvest height of $\geq$10 cm, relative yield response to N fertilizer in field sites with below normal precipitation didn't have a relationship with soil biological activity (Table 2.5 and 2.6; Fig. 2.15). At threshold yield efficiency of 5 kg DM kg^{-1} N, 13 sites were non responsive, one site was somewhat responsive and, the remaining three sites were responsive. Similarly, at threshold yield efficiency of 10 kg DM kg-1 N, 16 sites were non-responsive and one site was somewhat responsive. Actual yield response ranged from 0 to 805 kg DM ha^{-1} with a mean value of 304 kg ha^{-1}. N uptake response varied from 0 to 25 kg ha^{-1}. Economically optimum N rate varied from 0 to 86 kg ha^{-1} with a mean value of 20 kg ha^{-1} at threshold yield efficiency of 5 kg DM kg^{-1} N and 0 to 27 kg ha^{-1} with a mean value of 3 kg ha^{-1} at threshold yield efficiency of 10 kg DM kg^{-1} N, which were lower than values with near normal precipitation. There were 15 sites with below normal precipitation that were harvested at 5-10 cm. All 15 sites were non-responsive at both threshold yield efficiency of 5 and 10 kg DM kg^{-1} N. Actual yield response for these 15 sites varied from 8 to 408 kg DM ha^{-1} with a mean value of 145 kg DM ha^{-1}. Economically optimum N rate varied from 0 to 34 kg ha^{-1} with a mean value of 8 kg ha^{-1} at threshold yield efficiency of 5 kg DM kg^{-1} N and varied from 0 to 6 kg ha^{-1} at threshold yield efficiency of 10 kg DM kg^{-1} N with a mean value of <1 kg ha^{-1}.

Sites 16-08, 16-14 and 16-16 had high soil biological activity (497, 432, and 344 mg flush of CO_2 kg^{-1} soil, respectively) and didn't respond to N fertilization. No response to N fertilization could be attributed to soil biological activity or, low rainfall received by the sites or a combination of both. Sites 16-03, 16-15, 16-19, 16-22, 16-23 and 16-24 had high soil biological quality (444, 327, 390, 339, and 409 mg flush of CO_2 kg^{-1} soil, respectively) but had some response to N fertilization (Table 2.5 and 2.6). All of these sites had variably mixed tall fescue stands, the effect of which might have caused some species to have responded to additional N input. Sites 16-17, 16-18, 16-20, 16-21, and 16-27 had relatively lower soil biological activity (274, 186, 291, 224, and 214 mg flush of CO_2 kg^{-1} soil, respectively) but didn't respond to N fertilization most probably due to limiting rainfall. Sites 15-10, 15-22, and 16-07 responded to N fertilization despite of high soil biological activity (432, 367, 497 mg flush of CO_2 kg^{-1} soil, respectively) and limiting rainfall. This was surprising, as sites with high soil biological activity under normal precipitation were non-responsive to N fertilizer. Limited rainfall was expected to have limited the N fertilizer response. One possible reason behind this might be that since sites 15-10, 15-22, and 16-07 were >10-year old pastures, net N immobilization might have been a factor of which plants responded to N fertilization.

III. Above normal precipitation

Of the total of 57 field sites, nine had above normal precipitation. Relative yield response at threshold yield efficiency of 5 kg DM kg^{-1} N ranged from 0 to 5% with EONR ranging from 0 to 7 kg ha^{-1} N. None of the nine sites responded to N fertilization at threshold yield efficiency of 10 kg DM kg^{-1} N. Actual yield response ranged from 19 to 518 kg DM ha^{-1}. Relative yield response to N fertilizer of sites with above normal precipitation didn't show a relationship with soil biological activity (Table 2.5 and 2.6; Fig. 2.15).

Soil biological activity in the field sites with above normal precipitation varied from 185 to 498 mg flush of CO_2-C kg^{-1} soil. Lack of response to N fertilization may have been due to the high soil biological activity, as more than adequate precipitation should have led to some yield response if N were truly limiting.

Effect of management history on yield response of tall fescue to N fertilization

Management and fertilization history were evaluated for their role in yield and yield response of stockpiled tall fescue pastures. Most importantly, management and fertilization history could affect soil properties, particularly the soil biological activity, which would then influence yield and yield response of crops to N fertilization. In stockpiled tall fescue pastures, it would be useful to know if grazed pasture gives better yield than hayed pasture or if rotationally grazed pasture performs better than mob grazed pasture or so on. In our research, producers indicated variable management over time and we had many more grazed pasture than hayed. Also, both hayed and grazed pastures were not in the same location with similar soil properties. Future research with balanced number of tall fescue pastures with alternative management practices would be useful to assess the effect of management on yield response of stockpiled tall fescue to N fertilization.

Effect of nitrogen rate on forage dry matter, crude protein and moisture content

Nitrogen rate across 55 harvested sites was significantly correlated with crude protein (CP) and moisture content of stockpiled tall fescue (Fig. 2.16 and 2.17 respectively). Non responsive sites, somewhat responsive sites and responsive sites-all had a good correlation between CP and N rate ($r^2 = 0.94$, 0.97, and 0.97 respectively) and similarly moisture and N rate ($r^2 = 0.96$, 0.97, and 0.97 respectively). Rate comparison by least significant difference (lsd) test showed that crude protein was significantly different for 0, 45, 90 and 135 kg ha^{-1} N rates (Table 2.9). $\geq$10 cm tall fescue forage had higher crude protein compared to 5-10 cm forage (Fig. 2.16.b). Similarly, $\geq$10 cm tall fescue forage had higher field moisture compared to 5-10 cm forage (Fig. 2.17.b).

Dry matter production of stockpiled tall fescue increased with increase in N rate for any individual site that had a response to N fertilization (Appendix A). Tall fescue at $\geq$ 5 cm (addition of $\geq$10 and 5-10 cm) had more than double the DM as compared to $\geq$10 alone since the DM for 5-10 cm exceeded that of $\geq$10 cm for any individual site that responded to N fertilization (Appendix A).

Nitrogen uptake across all 55 harvested sites varied from 6 to 65 kg ha^{-1} (Appendix A). Nitrogen uptake ranged from 6 to 65 kg ha^{-1} for non-responsive sites, from 19 to 38 kg ha^{-1} for somewhat responsive sites and from 15 to 38 kg ha^{-1} for responsive sites. Nitrogen uptake and DM at 0 kg N ha^{-1} didn't correlate to soil biological activity (data not shown) as it did in greenhouse trials. Overall N use efficiency of $\geq$10 cm stockpiled tall fescue at 45 kg N ha^{-1} was 10%, at 90 kg N ha^{-1} was 4% and at 135 kg N ha^{-1} was 2%. Nitrogen use efficiency at 5-10 cm was 8, 3 and 1% at 45, 90 and 135 kg ha^{-1} respectively.

Relationship between net N mineralization and other soil biological properties

There was a strong positive correlation between the flush of CO_2 and net N mineralization ($r^2 = 0.59$, Fig. 2.18). Similarly, there was a strong positive correlation of net N mineralization with cumulative C mineralization and SMBC ($r^2 = 0.49$ and 0.52 respectively; Figure not shown). Total N was not correlated with net N mineralization but total C had a strong positive correlative with net N mineralization ($r^2= 0.59$). Particulate organic N and C had weak correlation ($r^2 = 0.12$ for both) with net N mineralization.

DISCUSSION

This study aimed to examine response of stockpiled tall fescue to N fertilization as affected by soil biological activity. There was a strong negative correlation of yield response (measured by relative yield response, EONR at threshold yield efficiencies of 5 and 10 kg DM kg^{-1} N, and N uptake response, and actual yield response) of stockpiled tall fescue at $\geq$10 cm with most of the active soil biological quality measures (the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC). Total C and N were weakly correlated and particulate organic C and N were not correlated to yield response of stockpiled tall fescue. Our hypothesis that biologically active soil N would alter yield response of stockpiled tall fescue to N fertilization was accepted.

Yield response of stockpiled tall fescue at height of 5-10 cm was relatively weaker compared to ≥10 cm height. This was likely due to new forage growth from fertilization at the top of the canopy than closer to the base. Active C and N fractions were more strongly correlated with yield response of stockpiled tall fescue to N fertilization compared to total C and N fractions because total C and N fractions also consists of a stabilized fraction, which is not generally associated with rapid microbial turnover of nutrients (Prasad-Dutta, 2010). Our finding that total N was weakly correlated to yield response supports the observation made by Hassink (1994) and Sellas et al. (1999) that the correlation between total N and N mineralization is weak. Total C, despite strong correlation with net N mineralization, had weaker correlation with yield response than active soil C and N fractions. It may be due to the fact that total C, as a whole didn't contribute to specific components of mineralization in the field during forage growth. Total soil N and C have been observed in the past as an index of N availability with mixed results (Schomberg et al., 2009). Other soil properties like initial inorganic N and humic matter were also regressed against yield response. Inorganic N was not significantly correlated with yield response. Plants take N in the inorganic form but inorganic N alone doesn't offer a complete assessment of soil fertility in relation to crop yield (Franzluebbers, 2016). Humic matter is routinely examined by current soil testing of North Carolina Department of Agriculture but our findings showed very weak correlation of humic matter with yield response of stockpiled tall fescue. This finding was similar to the semi-controlled study done by Pershing (2016). Humic matter being mostly recalcitrant possibly didn't reflect the mineralizable components of organic matter during the short-term field experiment.

The only active soil biological quality measures not correlated with yield response of stockpiled tall fescue to N fertilization were particulate organic N and C. This result was surprising because semi-controlled greenhouse experiments conducted before have shown a good correlation between yield and particulate organic N and C fractions, primarily in soils derived from long-term no-tillage cropping (Pershing, 2016). This finding also contradicts a field correlation of particulate organic C with yield in a rice-wheat-jute ecosystem (Majumder et al., 2007). Schomberg et al. (2009) found that particulate organic N and C were not as useful as C mineralization and SMBC in predicting mineralizable N. Particulate organic N and C in our study were also weakly correlated with net N mineralization, thereby questioning their reliability to predict N availability in soil.

Precipitation and other weather conditions in stockpiled fields may have greatly affected the yield and yield response of stockpiled tall fescue to N fertilization. This is because precipitation affects N uptake by plants (Nave et al., 2016). Some of the sites with above and normal precipitation despite of having low soil biological activity didn't respond to N fertilization. Type and intensity of rainfall could have affected plant growth. Above normal precipitation can cause lack of oxygen in the soil resulting in root loss and injury. Heavy rain can also compact soil which further decreases soil aeration. On the other hand, too little rainfall can damage plant tissues resulting in stunted growth and wilting. Low or no rainfall following urea fertilization can cause NH_3 volatilization (Koelliker and Kissel, 1988). NH_3 volatilization in our experiment was not seen a significant problem as: (i) N uptake and CP response was higher for higher N rate for the sites that responded to N fertilization, (ii) CP of the sites that responded to N fertilization at a particular N rate were similar to that measured by Teutsch (2005), and (iii) sites on the same location which had similar precipitation didn't have a similar response to N fertilization when the soil biological activity was different (Sites 15-07 and 15-08 were both in same location which received about the same amount of precipitation responded differently as their soil biological activity was different). The majority of the sites didn't respond to N fertilization as they exceeded some threshold level of soil biological activity.

Yield response of stockpiled tall fescue to N fertilization have been studied as a function of stockpiling initiation dates, N rates and timing of N fertilization (Rayburn et a., 1979; Gerrish et al. 1994). Present guidelines for N fertilization is based on date, current soil moisture, precipitation forecast, N prices and prices of alternative feeds (Poore et al., 2010) varying with environmental conditions (Teutsch et al. 2005). While substantial amount of research has examined the effect of N fertilization rate and timing, no research has examined soil biological quality effects on yield response of stockpiled tall fescue on N fertilization. Correlation of the flush of CO_2 with crop N uptake by bermudagrass was shown by Haney et al. (2001), but further calibration was deemed important (Franzluebbers, 2016). This research puts light on the fact that soil biological quality is an important factor to consider prior to fertilization of stockpiled tall fescue. This study also highlights the importance of testing soil biological activity in soil testing services.

We also looked for the effect of N rate on CP and moisture. Our finding that CP of stockpiled tall fescue significantly increased with the increase in N fertilization rate replicated the findings of Archer and Decker (1977), Rayburn et al. (1979) and Teutsch et al. (2005). Forage moisture content of stockpiled tall fescue also increased with N rate, suggesting a stimulation of growth by additional N. Similar trend was observed by Smika et al. (1965) in native pasture dominated by blue-grama, western wheatgrass, and needle and thread. Fertilizer increases water use efficiency of forages and may also improve root development facilitating moisture extraction at higher tensions and greater depths (Smika et al, 1961). Crude protein and moisture content were slightly lower for 5-10 cm tall fescue as compared to ≥10 cm. This was expected since ≥10 cm mostly consist of forage leaves while 5-10 cm is mostly stem with a higher proportion of dead leaves and unattached litter. Since, DM of stockpiled tall fescue was more than twice at ≥5 cm compared to ≥10 cm without much decline in the CP and moisture, it is profitable to use grazing height and cutting height of ≥5 cm. Grazing height of >5 cm has been found to increase forage utilization efficiency in stockpiled tall fescue (Lyons et al., 2016). While low cutting or grazing height might be problematic for regeneration of forages with shallow rooting system, tall fescue has deep rooting system (Nie et al., 2008; Franzluebbers and Stuedemann, 2009) and would not be expected to be limited in its regrowth, if sufficient recovery period were offered until later in springtime. Adequate soil fertility for regrowth would also need to be supplied.

A limitation of this study could be the use of only 0-10 cm soil depth for our soil analysis. Tall fescue is deep rooted and understanding the soil biological activity at multiple depths could help understand the effect of soil profile on response of stockpiled tall fescue to N fertilization. However, sampling deeper than 10 cm is a serious physical impediment in many years, as soil is often very dry following summer heat conditions, and obtaining a reliable sample from deeper in the profile might be problematic. Another limitation of our study was that we didn’t quantify the percentage of other forages mixed with tall fescue. The contamination of tall fescue pastures with other forages might have caused some variations in yield and yield response.

Among the soil biological properties measured, the flush of CO_2 was the best predictor of yield response with greater correlation with yield response compared to total N, total C, cumulative C mineralization, net N mineralization and SMBC. The flush of CO_2 correlated with net N mineralization and demonstrated a better predictive ability than net N mineralization possibly because of the short time period (3 days) required to measure the flush of CO_2 that minimized experimental error. The flush of CO_2: (i) is easy to measure; (ii) is accessible to many users; (iii) has the capacity to integrate physical, chemical and biological soil properties; (iv) is rapid; (v) is inexpensive and reproducible; and (v) correlates well to nutrient needs of crops and meeting environmental goals Franzluebbers (2016), thus could be used as an effective soil health tool.

Profitability is a key factor in making production and management decisions (Poore and Drewnoski, 2010). To make profitable decisions in a stockpiled tall fescue pasture, EONR was the most useful parameter as it combined yield with cost of hay and N fertilizer. EONR encourages the concept of economic return rather than maximum return. However, the cost of hay and N fertilizer has to be considered every time.

CONCLUSION

Fall stockpiled tall fescue had variable yield response to N fertilization, which was primarily a function of soil biological quality. Biologically active C and N fractions except particulate organic C and N were strongly negatively correlated with yield response of stockpiled tall fescue to N fertilization while total C and N fractions were weakly negatively correlated to N fertilization. The flush of CO_2 was the best predictor, while EONR was the best parameter reflecting yield response of stockpiled tall fescue to N fertilization. Forage at $\geq$10 cm had greater yield response to N fertilization, higher CP, and moisture than forage at 5-10 cm height, but with lower total DM harvested. Crude protein and moisture were not correlated with soil biological quality.

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Table 2.1. Location and management information for research fields

Year	Site no.[1]	State	County	Type of fescue	Age of Pasture (yrs.)	Management prior to experiment	Fertilization prior to experiment
2015	15-01	NC	Wake	E+[2]	>10	Rotationally grazed	NA[3]
2015	15-02	NC	Johnston	E+	>20	Mowed	Not usually but swine sludge in 2014
2015	15-03	NC	Wayne	Novel E+	1	Hay	NA
2015	15-04	NC	Pender	Novel E+	5	Rotationally grazed	Poultry litter in fall
2015	15-05	NC	Pender	Novel E+	5	Rotationally grazed	Poultry litter in fall
2015	15-06	NC	Montgomery	E+; mixed	>10	Mowed	
2015	15-07	NC	Durham	E+	>10	Rotationally grazed	NA
2015	15-08	NC	Durham	Novel E+	1	Occasionally grazed	NA
2015	15-09	NC	Granville	E+; mixed	28	Rotationally grazed + mowed	42-42-42 kg NPK/ha
2015	15-10	NC	Person	E+	>10	Rotationally grazed	NA
2015	15-11	NC	Rockingham	E+	>50	Occasionally grazed	56 kg N/ha in spring
2015	15-12	NC	Rockingham	E+; mixed	>25	Mowed	None
2015	15-13	NC	Surry	E+	14	Rotationally grazed	56-84 kg N/ha in fall
2015	15-14	NC	Surry	E+; mixed	14	Rotationally grazed	56-84 kg N/ha in fall
2015	15-15	NC	Ashe	E+; mixed	>40	Rotationally grazed	NA
2015	15-16	NC	Ashe	E+; mixed	>40	Occasionally grazed	NA
2015	15-17	NC	Rowan	Novel E+	1	Hay	None
2015	15-18	NC	Rowan	E+; mixed	>10	Mowed/occasionally grazed	None
2015	15-19	NC	Haywood	E+; mixed	>30	Rotationally grazed	Infrequently
2015	15-20	NC	Haywood	E+	>30	Rotationally grazed	Infrequently
2015	15-21	NC	Clay	E+	>40	Rotationally grazed	Poultry litter every 5 year

Table 2.1. Continued

2015	15-22	VA	Fauquier	E+	>10	Rotationally grazed	None
2016	16-01	WV	Monongalia	E+	NA	Hayed	PK and lime only
2016	16-02	WV	Monongalia	E+	NA	Hayed	None
2016	16-03	VA	Pulaski	E+; mixed	NA	Rotationally grazed	None
2016	16-04	VA	Carroll	E+	>50	Rotationally grazed	PK and lime only
2016	16-05	VA	Carroll	E+	>50	Rotationally grazed	PK and lime only
2016	16-06	VA	Carroll	E+	>50	Rotationally grazed	PK and lime only
2016	16-07	VA	Fauquier	E+	>50	Rotationally grazed	Limed
2016	16-08	VA	Madison	E+; mixed	>50	Rotationally grazed	Poultry litter
2016	16-09	VA	Augusta	E+; mixed	>50	Rotationally grazed	PK, lime and occasional N
2016	16-10	VA	Augusta	E+; mixed	>50	Rotationally grazed	None
2016	16-11	VA	Goochland	E+; mixed	>40	Rotationally grazed	Biosolids; 23 kg N last fall
2016	16-12	VA	Halifax	E+; mixed	16	Rotationally grazed	10:20:30 kg/ha NPK in fall
2016	16-13	NC	Ashe	E+	NA	Rotationally grazed	336 kg/ha/yr
2016	16-14	NC	Haywood	E+	28	Rotationally grazed	Inorganic NPK as needed
2016	16-15	NC	Clay	E+; mixed	>40	Rotationally grazed	Poultry litter every 5 years
2016	16-16	GA	Oconee	E+	>30	Occasionally grazed	NA
2016	16-17	GA	Oglethorpe	E+; mixed	40	Rotationally grazed	5 Mg/ha poultry litter
2016	16-18	GA	Oglethorpe	E+	3	Rotationally grazed	7 Mg/ha boiler litter
2016	16-19	GA	Wilkes	E+; mixed	>40	Rotationally grazed	Inorganic NPK as needed
2016	16-20	NC	Rowan	Novel E+	3	Hayed	Inorganic NPK as needed
2016	16-21	NC	Rowan	Novel E+	5	Hayed	Inorganic NPK as needed
2016	16-22	NC	Surry	E+; mixed	25	Rotationally grazed	50 kg/ha N in fall
2016	16-23	NC	Surry	E+; mixed	8	Rotationally grazed	15-15-15 kg/ha NPK in June
2016	16-24	NC	Surry	E+; mixed	>50	Rotationally grazed	25 kg/ha N in August
2016	16-25	NC	Stanly	E+	>50	Rotationally grazed	55 kg/ha UAN in fall
2016	16-26	NC	Randolph	E+	>10	Rotationally grazed	NA
2016	16-27	NC	Guilford	E+; mixed	15	Rotationally grazed	1.7 Mg/ha biosolids

Table 2.1. Continued

2016	16-28	NC	Rockingham	E+; mixed	>20	Rotationally grazed	None
2016	16-29	NC	Rockingham	E+; mixed	>5	Grazed + mowed	40 kg/ha UAN in spring
2016	16-30	NC	Person	E+	>20	Rotationally grazed	25 kg N/ha in fall
2016	16-31	NC	Durham	Novel E+	2	Rotationally grazed	73 kg/ha UAN
2016	16-32	NC	Granville	E+	>50	Rotationally grazed	NA
2016	16-33	NC	Pender	Novel E+	1	Rotationally grazed	2 Mg/ha litter after E+ renovation
2016	16-34	NC	Wayne	Novel E+	2	Hayed	Inorganic
2016	16-35	NC	Wayne	Novel E+	2	Hayed	Inorganic

[1] site no. are described in detail in appendix.

[2] Endophyte-infected wild type tall fescue.

[3] Not available

Table 2.2. Near infrared spectroscopy calibration for equation development for tall fescue forages.

Experiment year	Variable	No. of samples used for calibration (N)	Mean of N	Standard error of calibration (SEC)	SEC R-square	Standard error of cross validation (SECV)	SECV R-square	SEC as % Of Mean	SECV as % Of Mean	Math Treatment
2015	Nitrogen	90	2.183	0.034	0.994	0.047	0.989	1.539	2.148	1,10,10,1
2016	Nitrogen	276	2.064	0.044	0.990	0.051	0.987	2.127	2.451	1,10,10,1

Table 2.3. Eigen vectors of each key variables to predict yield response of stockpiled tall fescue to N fertilization for sites with near normal precipitation.

Response variable	Key predictors	Principal component 1	Principal component 2
Actual yield response	Flush of CO_2	0.70	0.70
	Soil microbial biomass carbon	0.70	-0.70
N uptake response	Flush of CO_2	0.70	0.70
	Soil microbial biomass carbon	0.70	-0.70
Relative yield response (@5 kg DM kg^{-1} N)	Flush of CO_2	0.99	-
Relative yield response (@10 kg DM kg^{-1} N)	Flush of CO_2	0.99	-
EONR (@5 kg DM kg^{-1} N)	Flush of CO_2	0.70	0.70
EONR (@ 10 kg DM kg^{-1} N)	Soil microbial biomass carbon	0.70	-0.70
	Flush of CO2	0.99	-

Table 2.4. Relative yield response, actual yield response, nitrogen uptake response and economically optimum nitrogen (EONR) rate for 2015 sites.

Site no.	Actual/ Normal Ppt[1]	Relative yield response (%)		Actual yield response (kg ha^{-1})	N uptake response (kg ha^{-1})	EONR (kg ha^{-1})	
		TYE 5 kg DM kg^{-1} N	TYE 10 kg DM kg^{-1} N			TYE 5 kg DM kg^{-1} N	TYE 10 kg DM kg^{-1} N
					Below normal precipitation		
15-10	0.00	19	0	511	14	26	0
15-22	0.17	35	15	491	21	43	14
					Near normal precipitation		
15-01	1.84	0	0	153	12	0	0
15-02	1.76	0	0	171	10	0	0
15-03	1.88	10	2	220	19	15	3
15-04	1.71	NA	NA	NA	NA	NA	NA
15-05	1.71	NA	NA	NA	NA	NA	NA
15-06	1.99	14	9	174	8	4	2
15-07	1.21	0	0	271	14	0	0
15-08	1.21	175	84	1238	25	115	29
15-09	1.77	29	13	587	19	54	13
15-11	1.29	0	0	63	6	0	0
15-12	1.29	34	12	572	16	34	0
15-17	1.71	83	33	741	20	50	12
15-18	1.71	0	0	226	10	1	0
15-21	1.39	1	0	491	7	2	0
					Above normal precipitation		
15-13	2.23	0	0	367	10	0	0

Table 2.4. Continued

15-14	2.23	5	0	353	14	7	0
15-15	2.31	5	0	63	4	4	2
15-16	2.31	0	0	2	5	1	0
15-19	2.40	4	0	3	11	4	0
15-20	2.40	2	0	4	7	4	0

[1] precipitation

Table 2.5. Relative yield response, actual yield response and economically optimum N rate (EONR) for 2016 sites

		Relative yield response (%)						EONR ($kg\ ha^{-1}$)			
		TYE 5 kg DM kg^{-1} N		TYE 10 kg DM kg^{-1} N		Actual yield response ($kg\ ha^{-1}$)		TYE 5 kg DM kg^{-1} N		TYE 10 kg DM kg^{-1} N	
Site no.	Actual/ Normal Ppt	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-20 cm	>10 cm	5-10 cm
						Below normal precipitation					
16-03	0.45	7	4	0	0	472	177	11	8	0	0
16-07	0.69	59	12	37	5	664	408	57	30	27	6
16-08	0.27	0	1	0	0	158	109	0	3	0	0
16-14	0.70	0	0	0	0	0	8	0	0	0	0
16-15	0.24	61	14	0	0	645	359	69	34	0	0
16-16	0.34	0	0	0	0	115	29	0	1	0	0
16-17	0.34	14	2	4	0	142	121	7	4	1	0
16-18	0.34	2	0	0	0	55	37	1	0	0	0
16-19	0.38	4	0	0	0	93	71	2	0	0	0
16-20	0.73	0	7	0	0	110	100	0	13	0	0
16-21	0.73	91	0	0	0	805	168	86	0	0	0
16-22	0.24	5	0	1	0	162	168	7	0	1	0
16-23	0.24	14	0	11	0	285	21	11	0	7	0
16-24	0.24	20	1	13	0	425	87	18	4	11	0
16-27	0.74	0	13	0	2	38	311	0	24	0	3
						Near normal precipitation					
16-01	1.02	0	0	0	0	239	183	0	0	0	0
16-02	1.02	0	0	0	0	179	99	0	0	0	0
16-04	0.98	29	2	13	0	489	263	20	3	6	0
16-05	0.98	0	0	0	0	105	65	0	0	0	0
16-06	0.98	6	0	0	0	246	47	5	0	0	0

Table 2.5. Continued

16-09	1.00	0	0	0	0	321	310	0	0	0	0
16-10	1.00	0	0	0	0	351	64	0	0	0	0
16-11	0.92	0	0	0	0	93	168	0	0	0	0
16-12	0.81	0	0	0	0	19	68	0	0	0	0
16-13	0.80	0	6	0	3	59	188	0	15	0	5
16-25	0.93	1	0	0	0	46	47	5	0	0	0
16-26	1.35	9	0	7	0	367	430	16	0	8	0
16-28	0.97	0	2	0	1	22	86	0	4	0	1
16-29	0.97	2	3	0	1	145	148	4	7	0	2
16-30	1.35	9	0	7	0	470	43	16	0	8	0
16-31	1.26	87	7	40	4	636	312	57	11	14	5
16-32	1.42	20	0	0	0	532	436	34	0	0	0
						Above normal precipitation					
16-33	2.00	0	5	0	1	99	155	0	7	0	10
16-34	2.32	0	6	0	4	213	176	0	6	0	3
16-35	2.32	0	10	0	6	74	461	0	27	0	12

Table 2.6. Nitrogen uptake response for 2016 sites.

Site no.	Actual/ Normal precipitation	N uptake response	
		>10 cm	5-10 cm
	Below normal precipitation		
16-03	0.45	16	9
16-07	0.69	22	14
16-08	0.27	5	6
16-14	0.70	0	0
16-15	0.24	17	12
16-16	0.34	9	5
16-17	0.34	6	6
16-18	0.34	2	2
16-19	0.38	3	3
16-20	0.73	6	6
16-21	0.73	18	9
16-22	0.24	7	8
16-23	0.24	9	2
16-24	0.24	14	9
16-27	0.74	4	8
	Near normal precipitation		
16-01	1.02	10	8
16-02	1.02	6	6
16-04	0.98	13	8
16-05	0.98	7	9
16-06	0.98	7	3
16-09	1.00	14	12
16-10	1.00	14	9
16-11	0.92	13	13

Table 2.6. Continued

16-12	0.81	3	5
16-13	0.80	4	10
16-25	0.93	4	2
16-26	1.35	11	10
16-28	0.97	2	6
16-29	0.97	8	6
16-30	1.35	17	7
16-31	1.26	14	11
16-32	1.42	15	15
	Above normal precipitation		
16-33	2.00	8	8
16-34	2.32	7	6
16-35	2.32	10	19

Table 2.7. Coefficients of determination from non-linear regression models of soil properties versus yield response parameters, crude protein and field moisture for ≥10 cm harvest height.

	Fertilizer response		Economically Optimum N rate				
Soil biological quality measures	TYE of 5 kg DM per kg of N	TYE of 10 kg DM per kg of N	TYE of 5 kg DM per kg of N	TYE of 10 kg DM per kg of N	Actual response to N	Crude protein	Field moisture
Flush of CO_2	0.86^{***}	0.86^{***}	0.63^{***}	0.74^{***}	0.59^{***}	NS	NS
Cumulative C mineralization	0.76^{***}	0.73^{***}	0.53^{***}	0.63^{***}	0.53^{***}	NS	NS
Net N mineralization	0.80^{***}	0.81^{***}	0.60^{**}	0.70^{***}	0.56^{**}	NS	NS
Soil microbial biomass C	0.65^{***}	0.59^{***}	0.53^{***}	0.53^{***}	0.46^{***}	NS	NS
Particulate organic N	NS	NS	NS	NS	NS	NS	NS
Particulate organic C	NS	NS	NS	NS	NS	NS	NS
Total C	0.45^{***}	0.42^{***}	0.42^{***}	0.40^{***}	0.30^{***}	NS	NS
Total N	0.34^{***}	0.30^{**}	0.37^{***}	0.31^{**}	0.28^{***}	NS	NS
Inorganic N	NS	NS	0.13^{*}	NS	0.12^{*}	NS	NS
Available N	0.75^{***}	0.76^{***}	0.58^{***}	0.68^{***}	0.50^{***}	NS	NS
Humic matter	NS	NS	NS	NS	NS	NS	NS
Cation exchange capacity	NS	NS	NS	NS	NS	NS	NS
Potassium	NS	NS	NS	NS	NS	NS	NS
Calcium	NS	NS	NS	NS	NS	NS	NS
Magnesium	NS	NS	NS	NS	NS	NS	NS
pH	NS	NS	NS	NS	NS	NS	NS
Phosphorus	NS	NS	NS	NS	NS	NS	NS
Sulfur	NS	NS	NS	NS	NS	NS	NS
Manganese	NS	NS	NS	NS	NS	NS	NS

Table 2.7. Continued

Zinc	NS	NS	NS	NS	NS	NS	NS
Copper	NS	NS	NS	NS	NS	NS	NS

[*]significant at 0.05 probability level

[**]significant at 0.01 probability level

[***]significant at 0.001 probability level
NS not significant

Table 2.8. Coefficients of determination from linear regression models of soil properties versus yield response parameters for $\geq$10 cm harvest height

	Fertilizer response			Economically Optimum N rate	
Soil properties	TYE of 5 kg DM per kg of N	TYE of 10 kg DM per kg of N	Actual yield response	TYE of 5 kg DM per kg of N	TYE of 10 kg DM per kg of N
Humic matter	0.04*	0.03*	NS	0.07*	0.03*
Cation exchange capacity	0.16*	0.13*	0.25*	0.18*	0.17*
Potassium	0.09*	0.10*	0.10*	0.08*	NS
Calcium	0.13*	0.10*	0.24*	0.16*	0.10*

*significant at 0.05 probability level

**significant at 0.01 probability level

***significant at 0.001 probability level

NS not significant

Table 2.9. Crude protein and field moisture of stockpiled tall fescue across 57 sites of study

	Crude protein (%)			Forage moisture (%)		
N rate	>10 cm harvest ht. (2015 and 2016 sites)	>10 cm harvest ht. (2016 sites)	5-10 cm harvest ht. (2016 sites)	>10 cm harvest ht. (2015 and 2016 sites)	>10 cm harvest ht. (2016 sites)	5-10 cm harvest ht. (2016 sites)
0	11.5^{a}	11.0^{a}	10.8^{a}	55.1^{a}	54.0^{a}	53.1^{a}
45	12.7^{b}	12.3^{b}	11.8^{b}	59.2^{b}	57.2^{b}	55.4^{b}
90	13.6^{c}	13.2^{c}	12.6^{c}	60.0^{bc}	58.2^{b}	56.2^{bc}
135	14.6^{d}	14.3^{d}	13.3^{d}	61.3^{c}	60.1^{c}	57.2^{c}

Means with the same letter are not significantly different

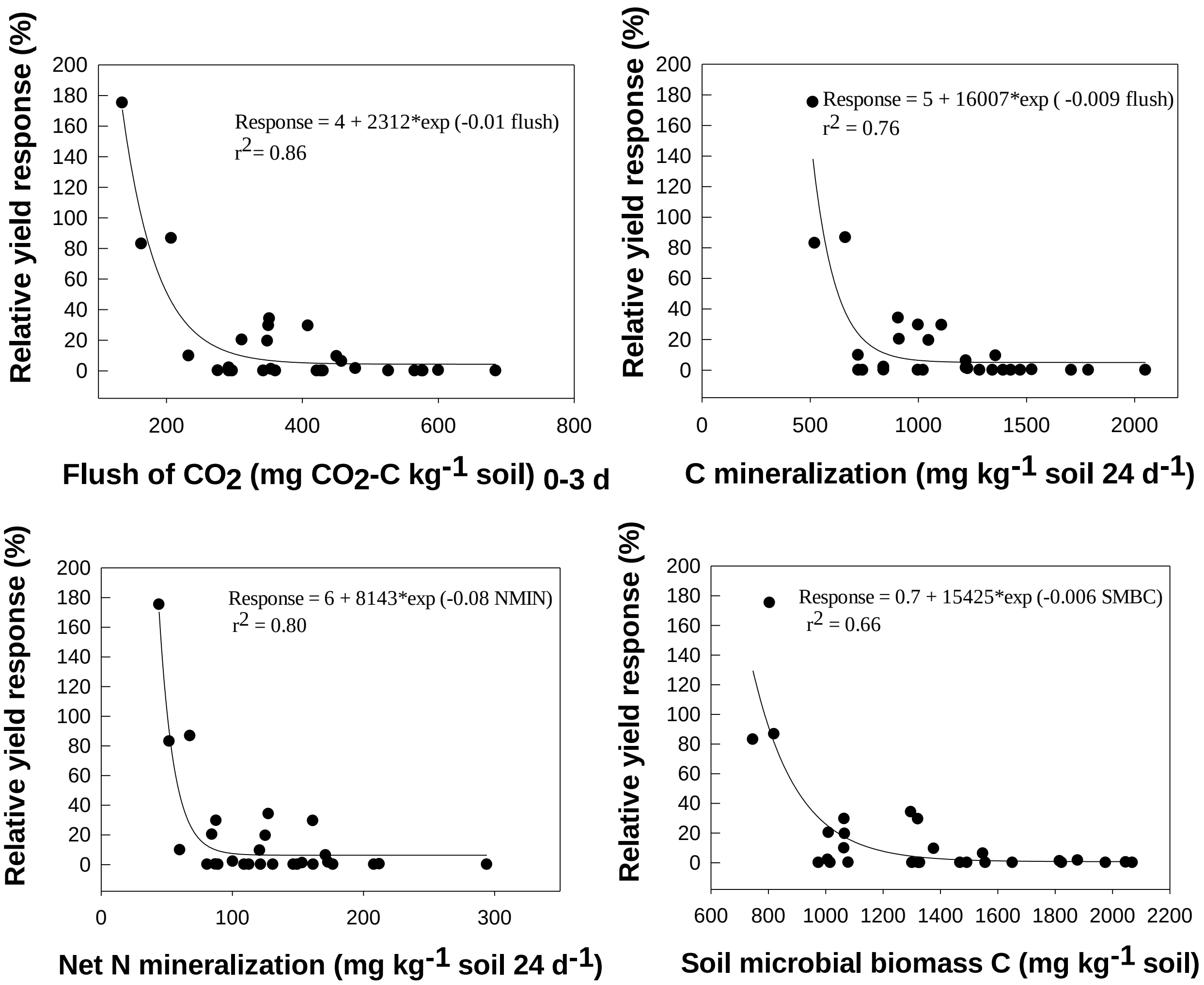

Figure 2.1. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 5 kg DM kg^{-1} N as affected by active soil biological quality measures (near normal precipitation).

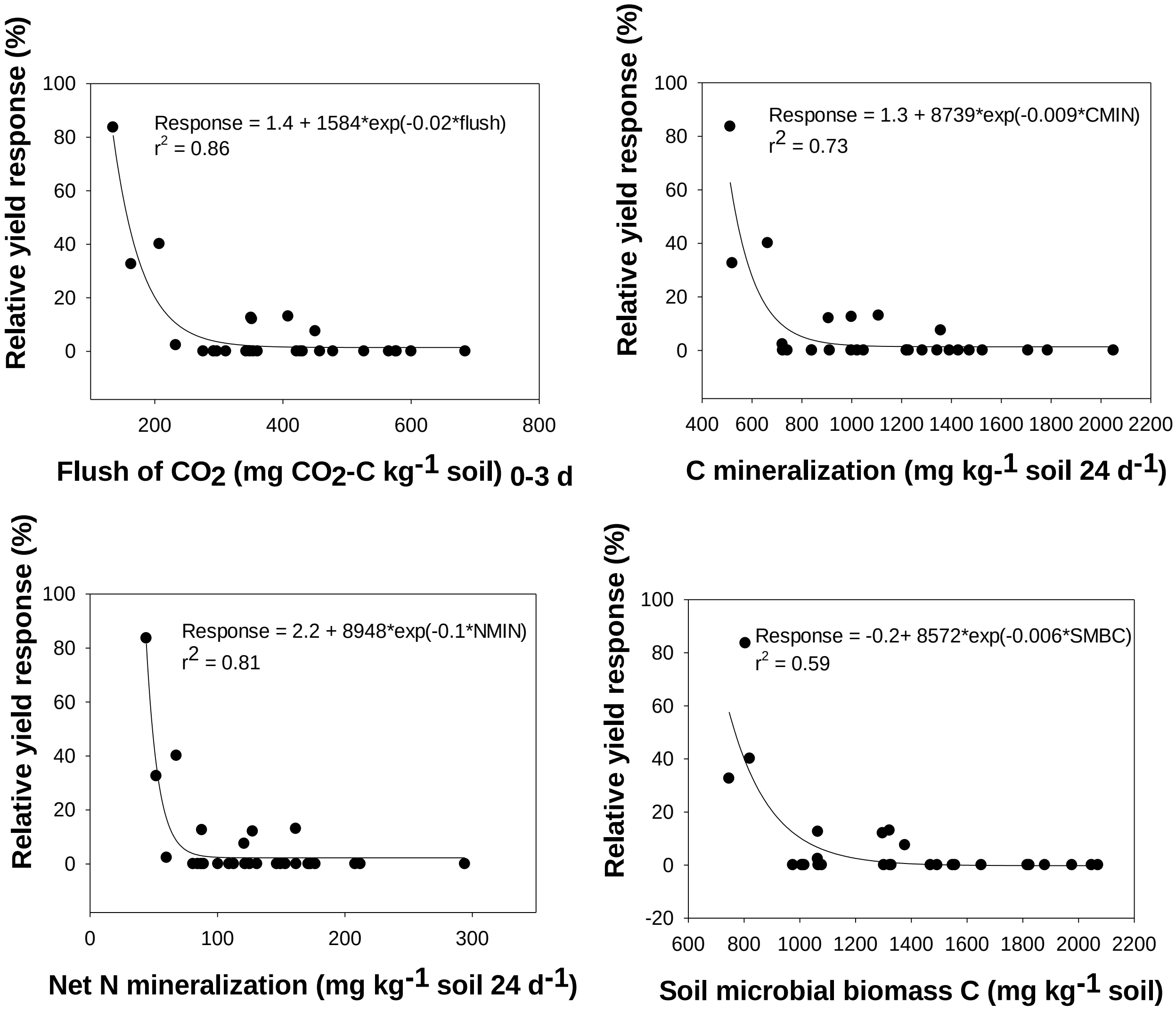

Figure 2.2. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 10 kg DM kg^{-1} N as affected by active soil biological quality measures (near normal precipitation).

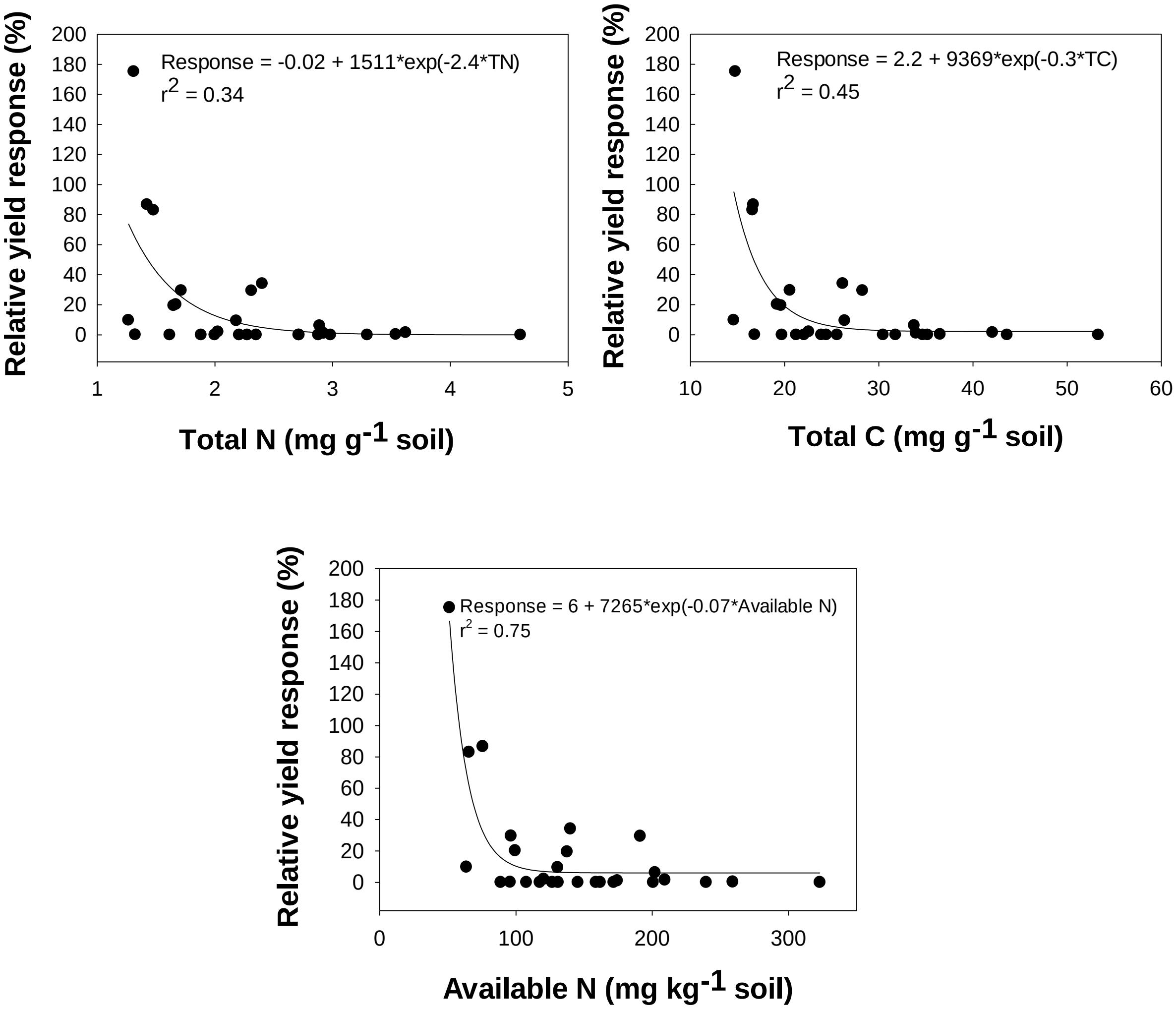

Figure 2.3. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 5 kg DM kg^{-1} N as affected by total nitrogen, total carbon and available nitrogen (near normal precipitation).

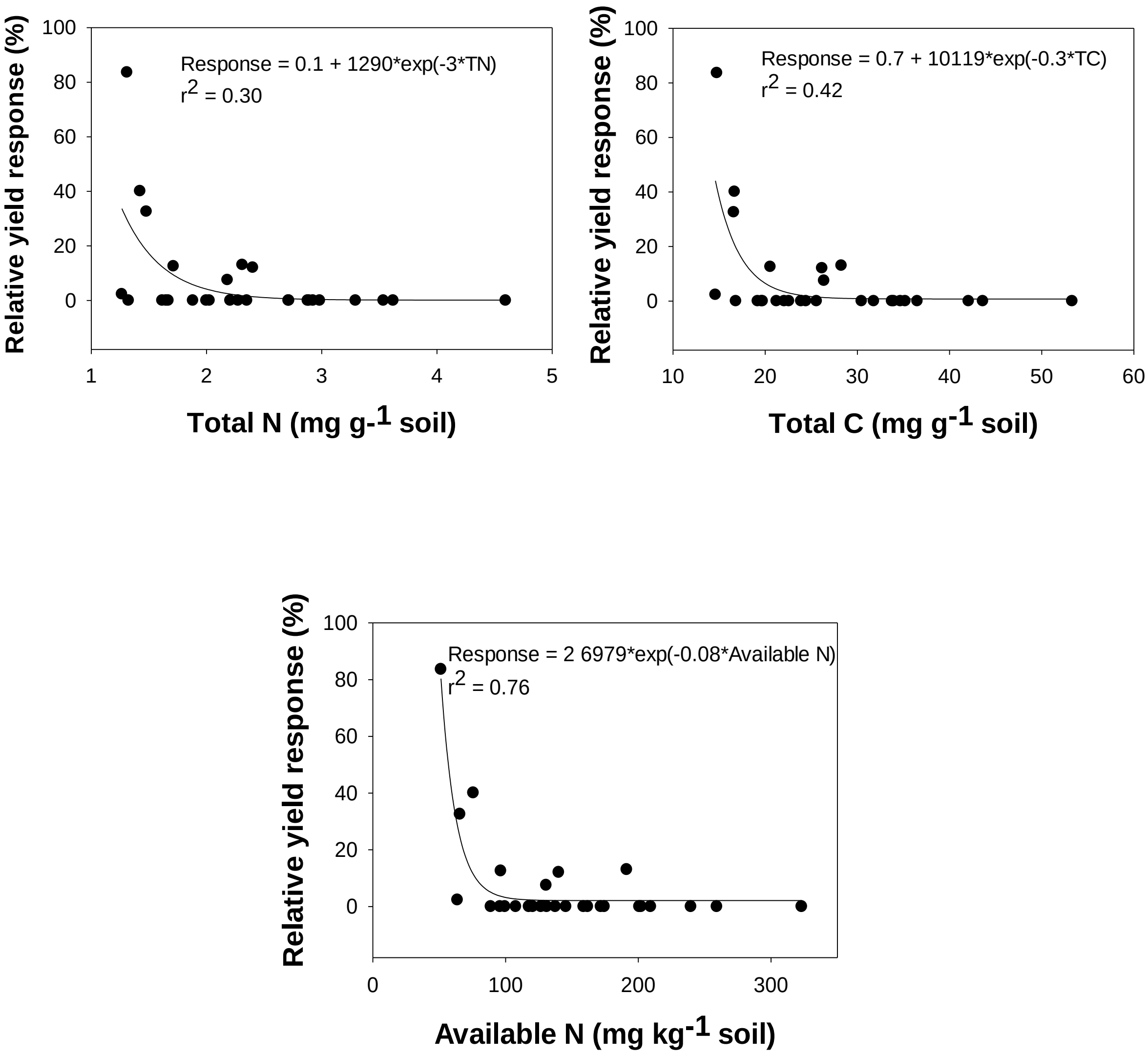

Figure 2.4. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 10 kg DM kg^{-1} N as affected by total nitrogen, total carbon and available nitrogen (near normal precipitation).

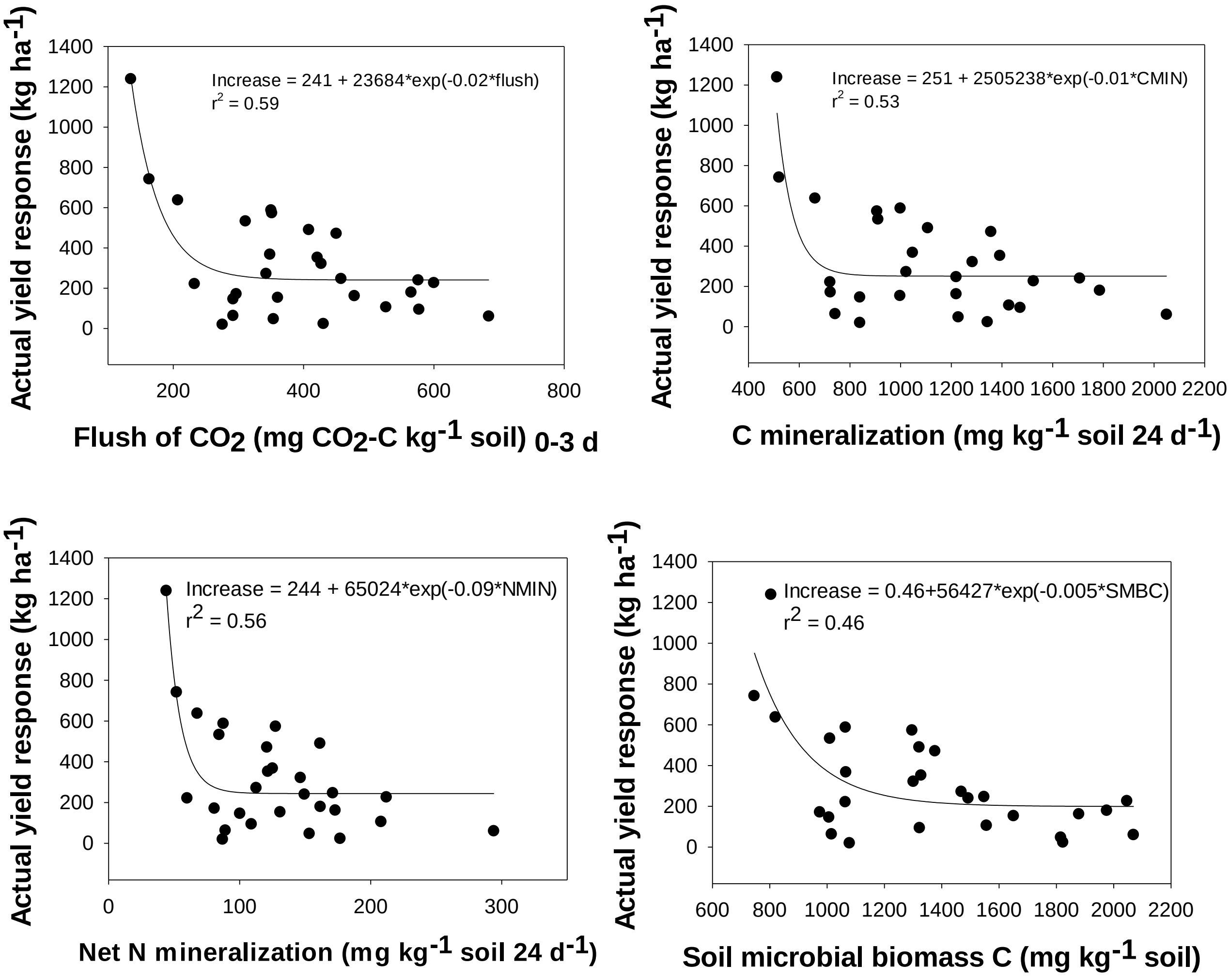

Figure 2.5. Actual yield response of stockpiled tall fescue to nitrogen fertilization as affected by active soil biological quality measures (near normal precipitation).

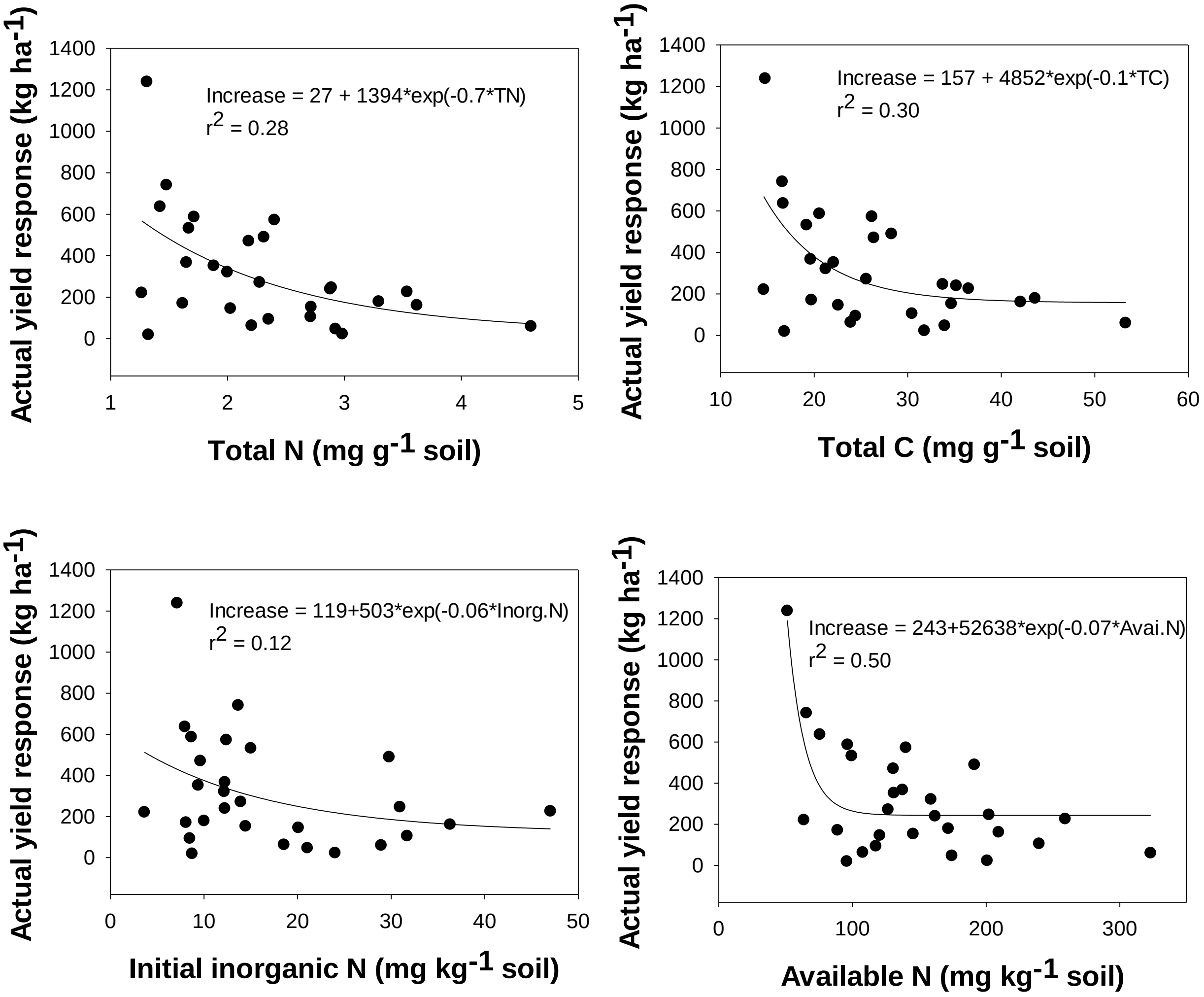

Figure 2.6. Actual yield response of stockpiled tall fescue to nitrogen fertilization as affected by total nitrogen, total carbon, initial inorganic nitrogen and available nitrogen (near normal precipitation).

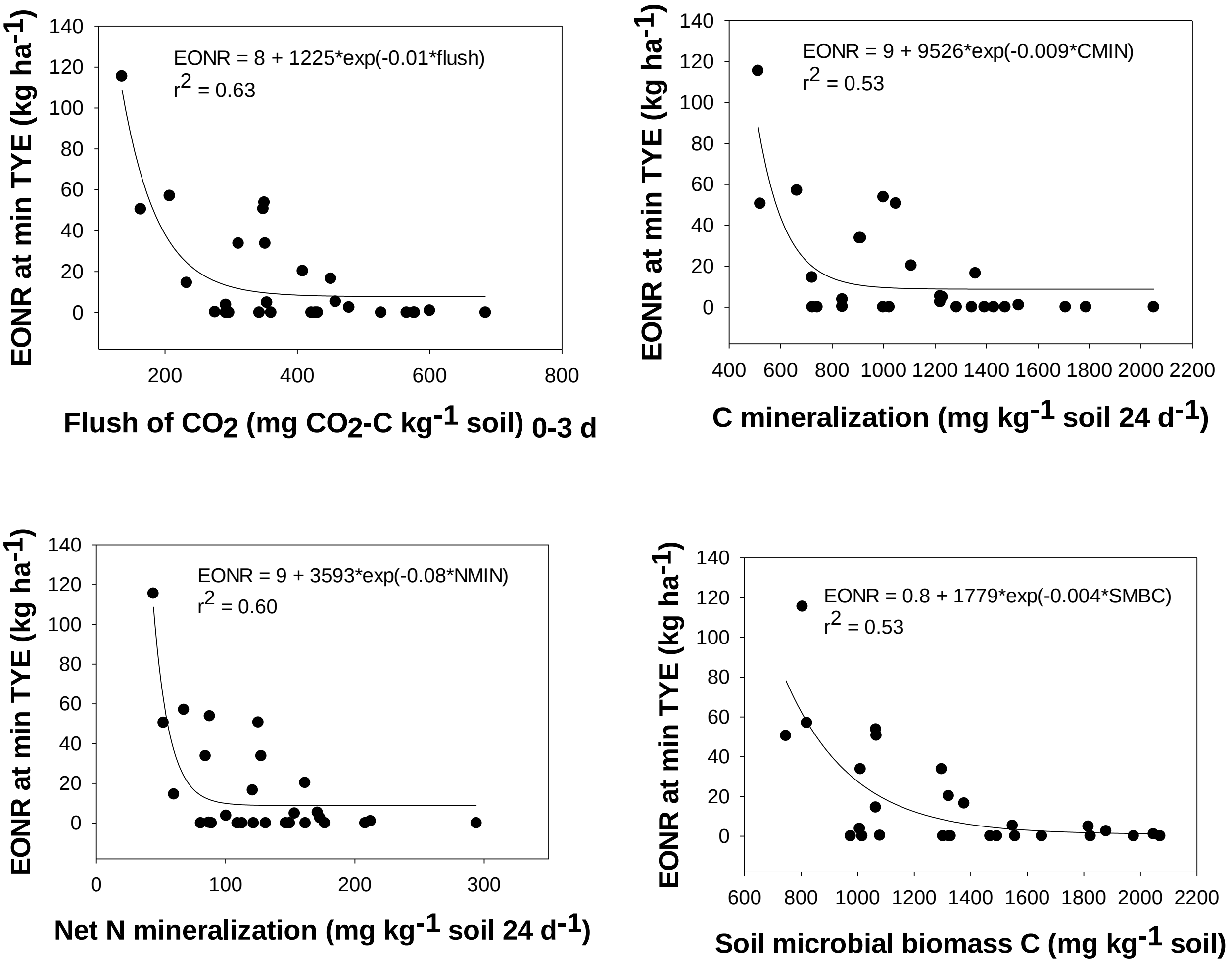

Figure 2.7. Economically optimum nitrogen rate for stockpiled tall fescue at threshold yield efficiency of 5 kg DM kg^{-1} N as affected by soil biological quality measures (near normal precipitation).

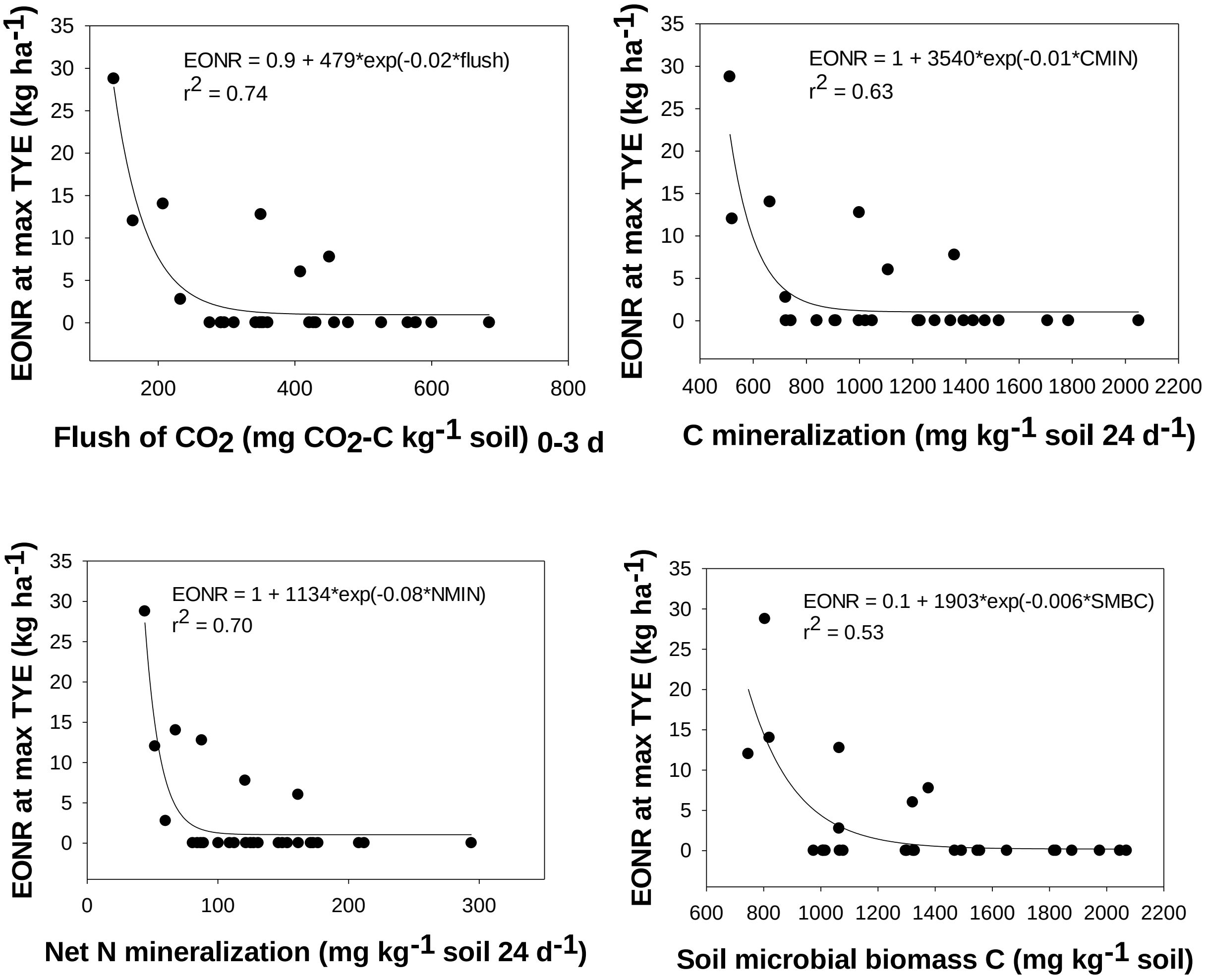

Figure 2.8. Economically optimum nitrogen rate for stockpiled tall fescue at threshold yield efficiency of 10 kg DM kg^{-1} N as affected by active soil biological quality measures (near normal precipitation).

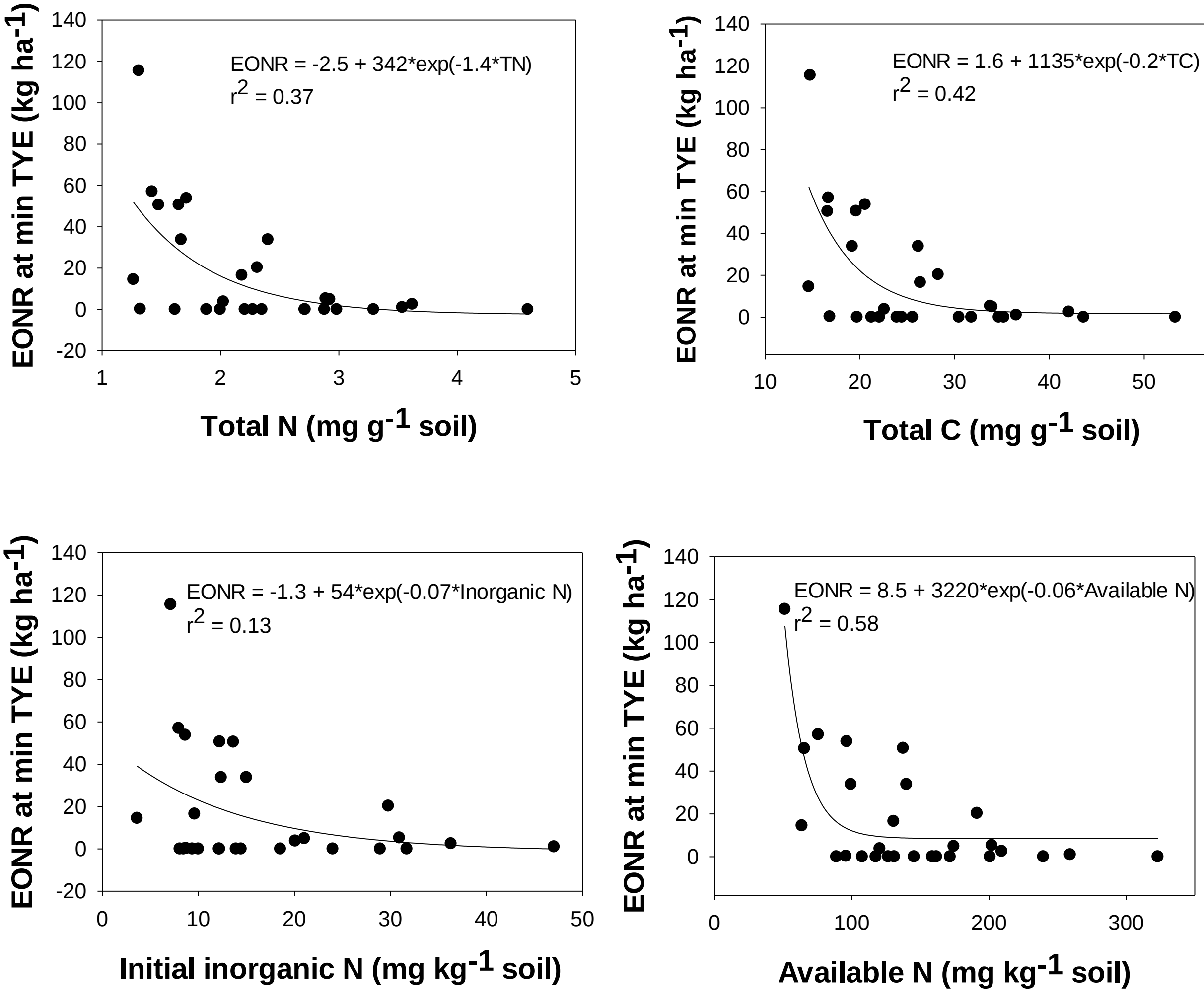

Figure 2.9. Economically optimum nitrogen rate for stockpiled tall fescue at threshold yield efficiency of 5 kg DM kg^{-1} N as affected by total nitrogen, total carbon, initial inorganic nitrogen and available nitrogen (near normal precipitation).

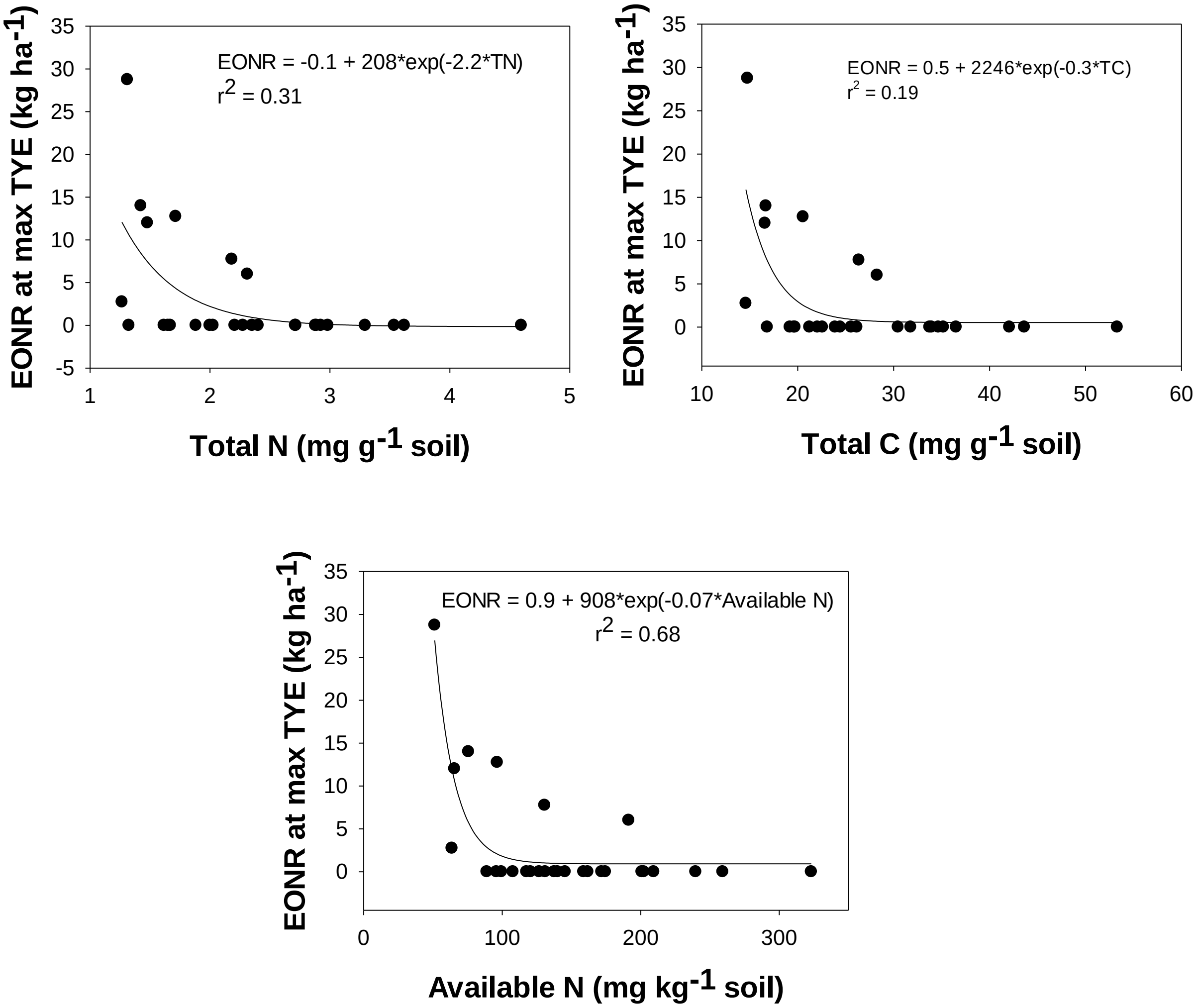

Figure 2.10. Economically optimum nitrogen rate for stockpiled tall fescue at threshold yield efficiency of 10 kg DM kg^{-1} N as affected by total nitrogen, total carbon, nitrogen and available nitrogen (near normal precipitation).

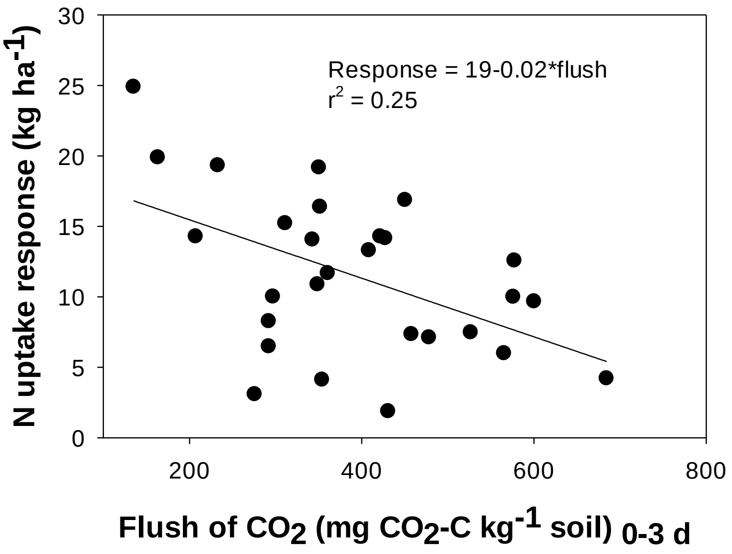

Figure 2.11. Nitrogen uptake response for stockpiled tall fescue as affected by the flush of CO_2 (near normal precipitation).

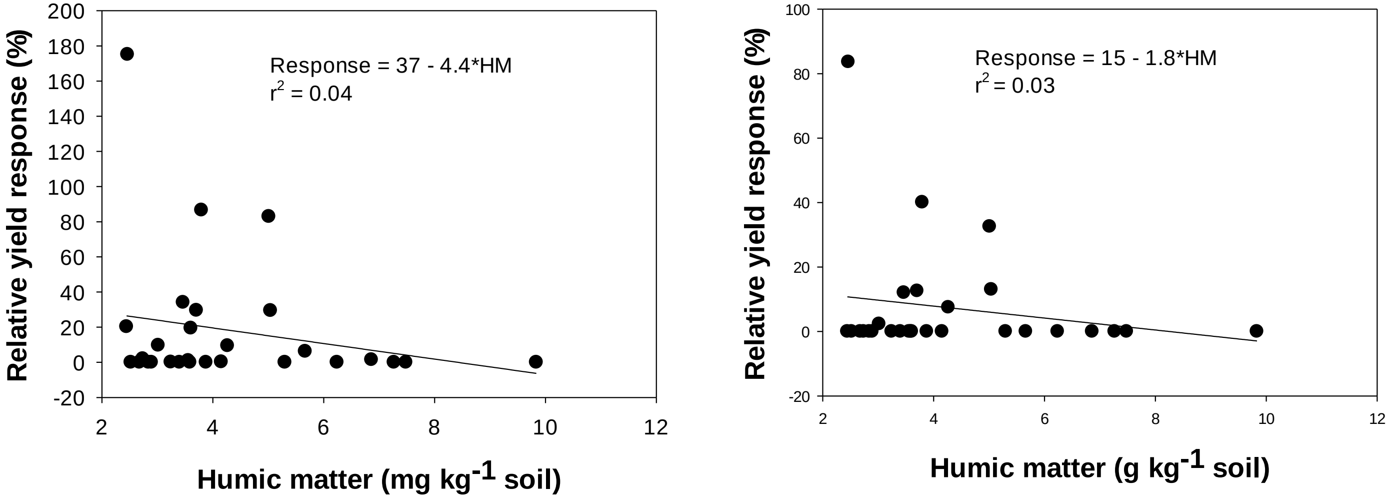

Figure 2 a.

Figure 2b.

Figure 2.12. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 5 kg DM kg^{-1} N (2.a.) and 10 kg DM kg^{-1} N (2. b.) as affected by humic matter (near normal precipitation).

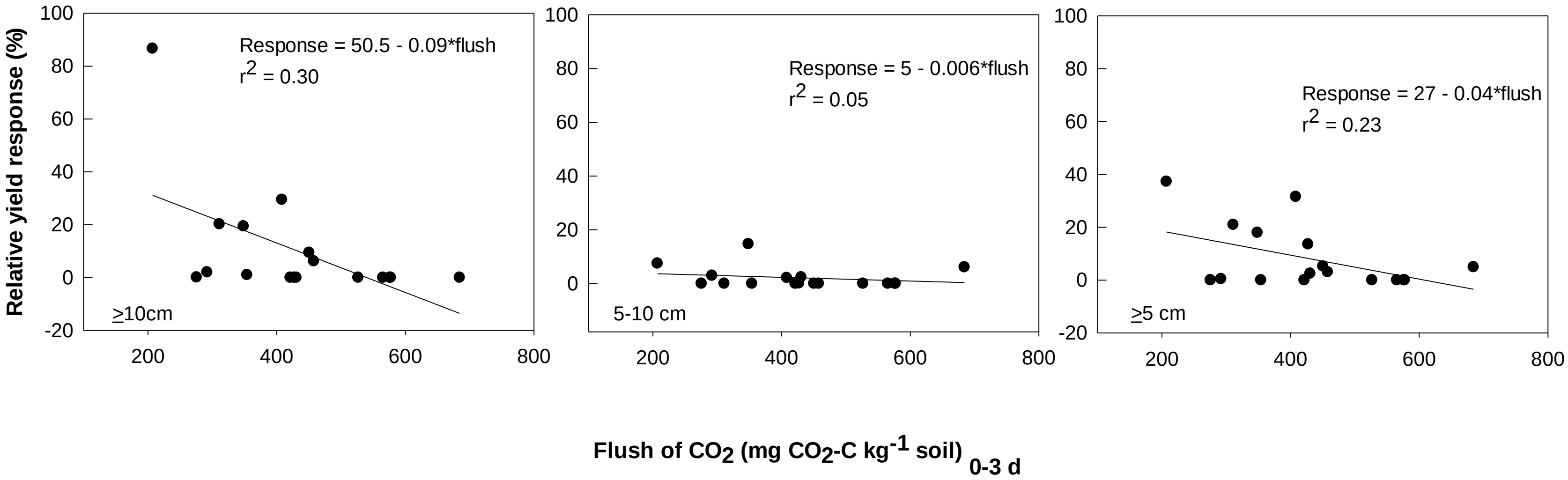

Figure 2.13. a. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at 3 different harvest heights at threshold yield efficiency of 5 kg DM kg^{-1}N as a function of the flush of CO_2 (2016 sites with near normal precipitation).

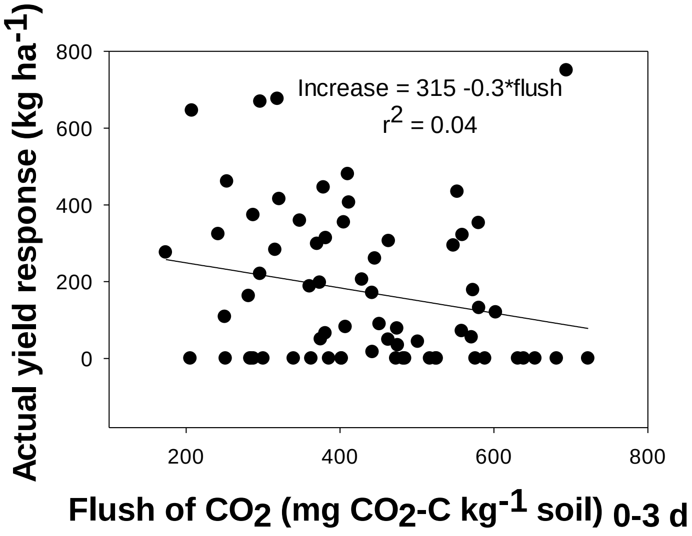

Figure 2.14. Actual yield response of stockpiled tall fescue to nitrogen fertilizer at 5-10 cm harvest height at threshold yield efficiency of 5 kg DM kg^{-1}N as a function of the flush of CO_2 (2016 sites with near normal precipitation).

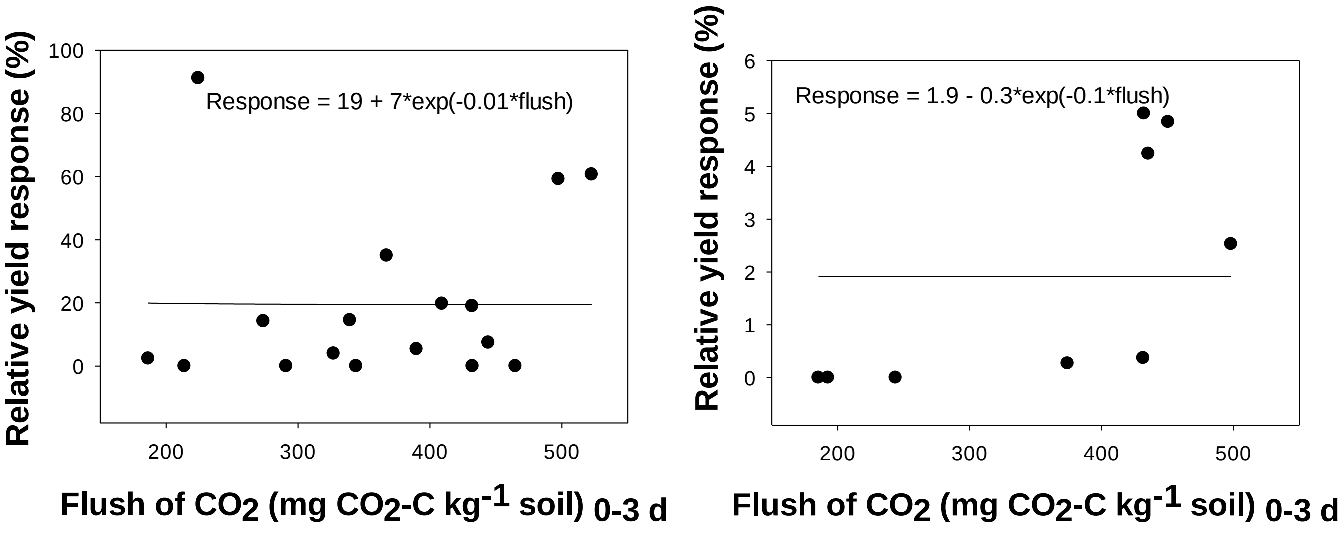

Fig. 2.13. a

Fig. 2.13.b

Figure 2.15. Relative yield response of stockpiled tall fescue to nitrogen fertilizer at threshold yield efficiency of 5 kg DM kg^{-1} N as a function of the flush of CO_2 for: (a) fields with below normal precipitation (b) fields with above normal precipitation

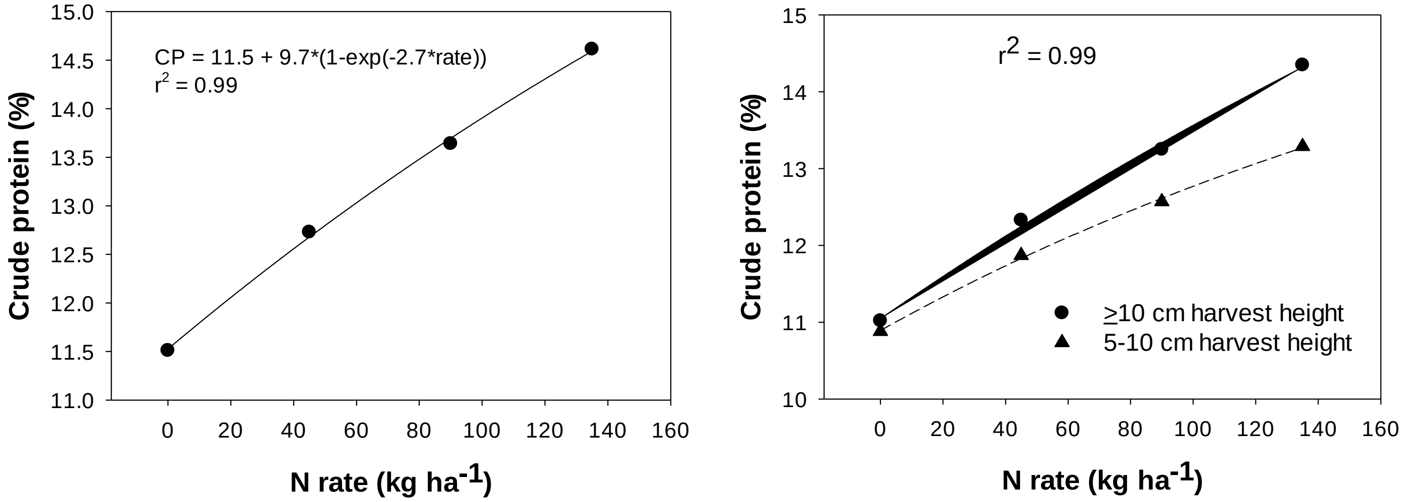

Fig. 2.14.a

Fig. 2.14.b

Figure 2.16. Crude protein of stockpiled tall fescue as a function of nitrogen rate for: (a) 2015 and 2016 sites together (≥10 cm) (b) ≥10 cm and 5-10 cm harvest height of 2016 sites

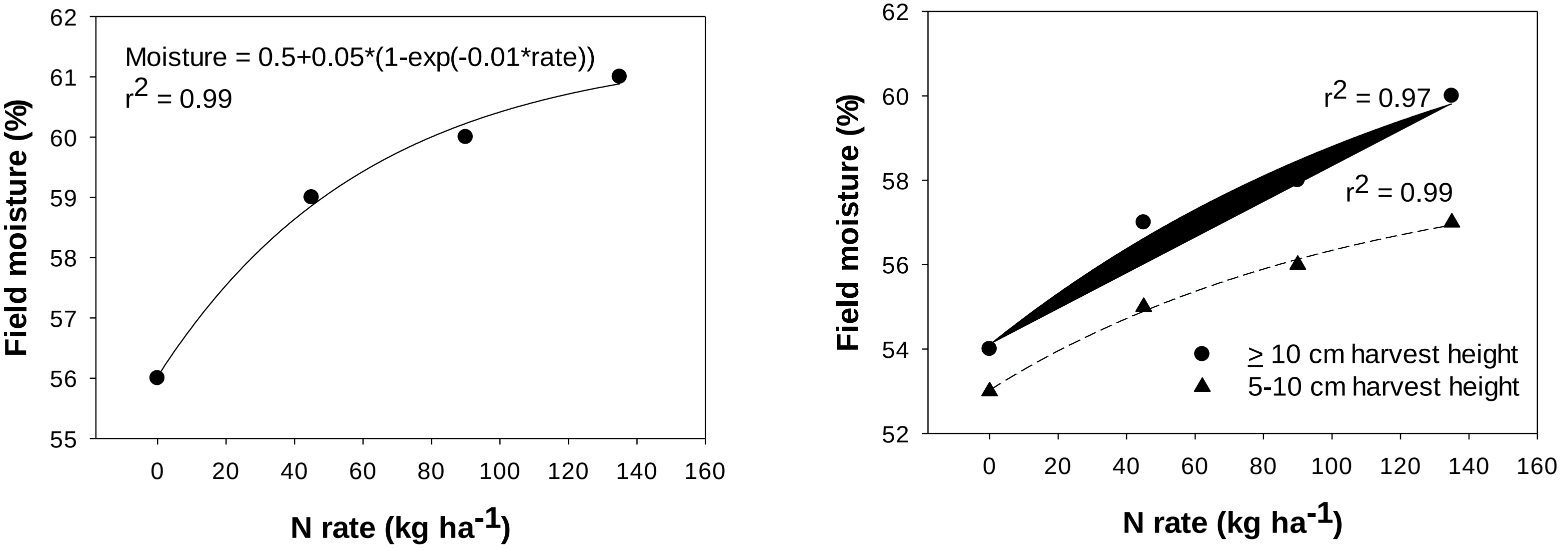

Fig. 2.17.a

Fig. 2.17.b

Figure 2.17. Field moisture of stockpiled tall fescue as a function of nitrogen rate for: (a) 2015 and 2016 sites together (≥10 cm) (b) >10 cm and 5-10 cm harvest height of 2016 sites

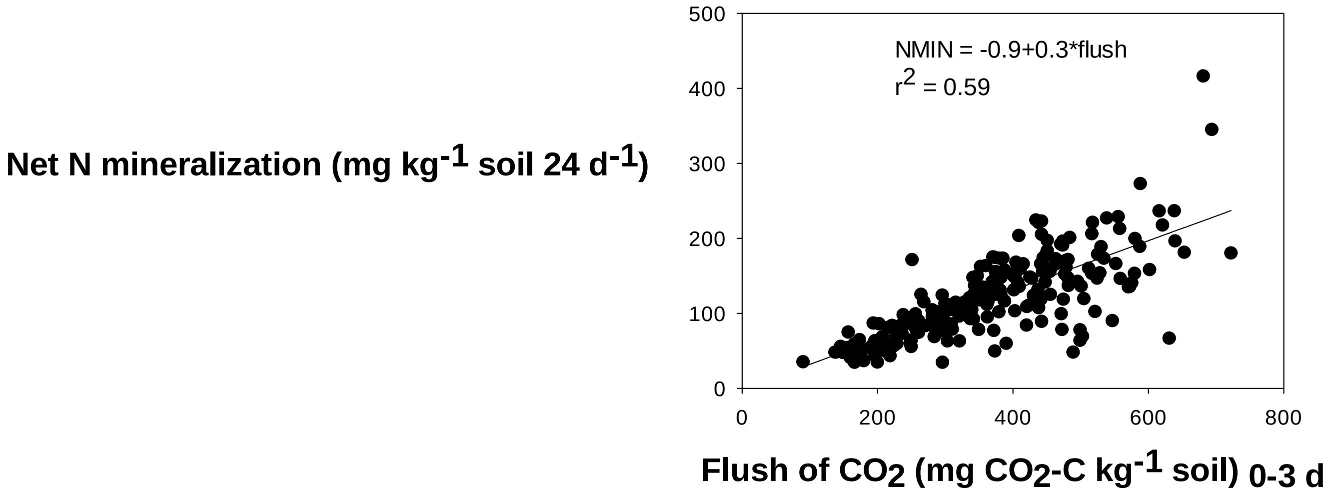

Figure 2.18. Relationship between Net N mineralization and the flush of CO_2 for 2015 and 2016 sites.

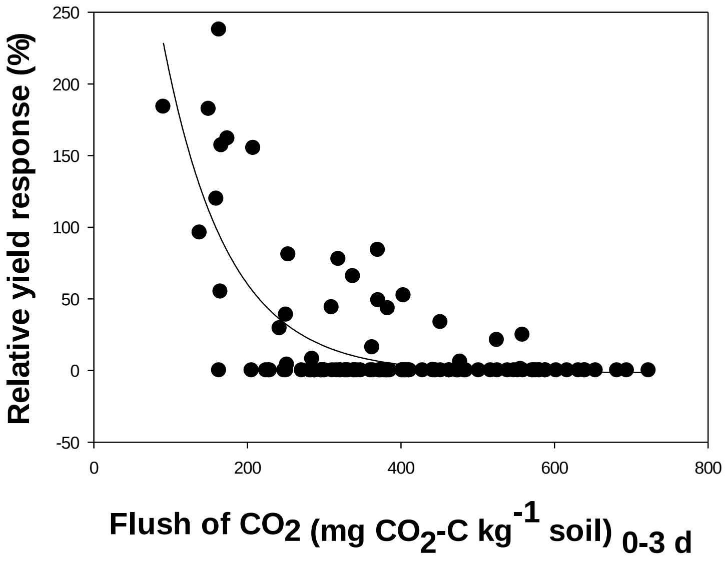

Figure 2.19. Relative yield response at threshold efficiency of 5 kg DM kg^{-1} N as affected by soil biological activity (near normal precipitation sites with all four replication included for each sites).

CHAPTER 3

BIOASSAY OF PLANT GROWTH FROM TALL FESCUE PASTURE SOILS VARYING IN C AND N FRACTIONS

ABSTRACT

Biologically active soil carbon (C) and nitrogen (N) fractions are important attributes of healthy soil. As a bioassay, we hypothesized that active soil C and N fractions would relate with plant dry matter (DM) production and N uptake from unamended soil. Four replicate soil samples (0-10-cm depth) from 57 tall fescue fields in North Carolina, Virginia, West Virginia, and Georgia were collected in either August 2015 or August/September 2016 for evaluation of soil C and N mineralization through aerobic incubation at 25°C and 50% water-filled pore space for up to 24 days. A greenhouse trial was conducted each year with triplicate subsamples of each field replicate utilized as a medium for sorghum-sudan grass growth during 6 weeks. Dry matter of sorghum-sudan grass was highly correlated with the flush of CO_2 for both years (r^2 = 0.66 and 0.76, respectively). Dry matter was also highly correlated with cumulative C mineralization (r^2= 0.59 and 0.63), net N mineralization (r^2=0.62 and 0.64), and soil microbial biomass C (r^2 = 0.58 and 0.55) in both trials. Plant N uptake was correlated with the flush of CO_2 (r^2 = 0.77 and 0.72), cumulative C mineralization (r^2= 0.72 and 0.81), net N mineralization (r^2 = 0.58 and 0.68), and soil microbial biomass C (r^2 = 0.70 and 0.37) in both trials. Particulate organic C and N did not relate to plant DM production and N uptake in either greenhouse trial. Cumulative C mineralization, soil microbial biomass C (SMBC) and total soil N were key predictors of plant DM, and the flush of CO_2, cumulative C mineralization, and total soil N were key predictors of plant N uptake in Greenhouse Trial 1. The flush of CO_2 and cumulative C mineralization were key predictors of plant DM and N uptake in Greenhouse Trial 2. This study confirmed the validity of measuring soil biological quality as a soil testing tool to predict soil N availability and potential plant growth.

INTRODUCTION

Soil biological activity is a key indicator of soil health and plant productivity (Franzluebbers, 2016). Although plant growth is dependent on inorganic N content in soil, soil biological activity serves to supply N through mineralization.

Soil organic matter has been evaluated for estimating potential mineralization of nutrients in the past but a huge fraction of soil organic matter could be resistant to mineralization. Active soil biological activity has to be measured in order to understand how minerals will be supplied during their growing stages. N mineralization can be measured by active C and N fractions in relation with the DM production and N uptake of plants.

Our hypothesis was that active C and N fractions in the soil will relate to plant dry matter production and N uptake. Current soil testing labs do not have an adequate test for differentiating N recommendations prior to fertilization. Understanding soil attributes that estimate potential N mineralization could significantly improve soil and nutrient management.

The objective of conducting a greenhouse experiment was to relate plant growth and N uptake to N supplying capacity of soil. Soil biological quality measures used in this study were the flush of CO_2, cumulative C mineralization, net N mineralization, soil microbial biomass C (SMBC), and particulate organic N and C along with total soil N and soil organic C. Other physical and chemical properties were also analyzed for their effect on plant DM production and N uptake.

MATERIALS AND METHODS

Preparation of tubes and soil weighing

Soil samples (n = 88) previously obtained from 22 different fields in August 2015 were evaluated in Greenhouse Trial 1. Ray Leach containers (Ray leach RLC4 cells “cone-tainers”), approximately 160 mL, were used for the analysis. One large cotton ball was placed in the bottom of each tube to prevent loss of soil. Tubes were filled to approximately 2 cm of the top, gently tamped to settle the soil, and net soil weight (g) was recorded. Each soil was replicated 3 times resulting in a total of 264 tubes in Trial 1.Soil samples (n = 140) previously obtained from 35 different fields in August 2016 were replicated three times resulting in a total of 420 samples in Trial 2.

Germination and Planting

Greenhouse Trial 1 was conducted in June/July 2016 and Trial 2 was conducted in February/March 2017. Approximately 24 hours prior to planting, sorghum-sudan seeds as test crop were placed on moistened paper towels (top and bottom) in a shallow plastic tray, covered with a top tray to prevent drying, and placed in an incubator at 25°C. The evening prior to planting, 10 mL of water was added to the surface of each soil tube. In the morning just prior to planting, another small amount of water was added to the surface to further moisten the soil. Five germinated seeds were placed on the top of each soil. Care was taken to gently press the seed into the moist soil, and approximately 20 g of finely grained sand was added to cover the seeds. After all seeds were planted and sand added, an additional 5 mL of water was applied to the sand. Racks were covered with lab bench paper to reduce evaporation until seedlings appeared. All tubes remained at room temperature, with additional water (5 mL) applied daily to the tops of each tube until three days post planting at which time all racks were transferred to the greenhouse. Artificial light was provided early morning and late afternoons to provide a total of 12 hours of daylight per day. Every day or two as needed, all greenhouse racks were placed in watering trays for approximately 15 minutes and allowed to absorb water through the bottom of each tube. One tray of samples was used to monitor water usage (Table 3.1). A weight was obtained dry and again after thoroughly watering, to estimate maximum water uptake. Once this amount was determined, water levels were maintained at or above 40% of the maximum water level. Watering was performed using watering trays when levels fell to near 40% saturation, but additionally all tubes were watered from the top (approximately 2 dispenses of 10 mL each) on Monday of each week post planting. Water trays were emptied of excess water after each watering.

Harvesting

All plant tissue was harvested after six weeks of growth. Number of plants was noted per greenhouse tube. Only a few seedlings did not develop, as the average number of plants per tube was five for both trials. All plant tissue was then cut and placed in a coin envelope, oven dried to constant weight (55 °C, 48 hours) and dry weight recorded. Plant dry matter was then expressed as milligrams dry matter per gram of soil to account for difference in soil weights attributed by soil texture and bulk density.

Plant nitrogen determination

Harvested sorghum-sudangrass samples were oven dried combining the three replications together (55˚C, $\geq$ 3 d), ground to pass a 1 mm screen in Udy mill, thoroughly mixed, and a subsample subjected to evaluation for N concentration using near infrared spectroscopy (NIRS). All samples were scanned in a model 5000 NIRS with WinISI version 1.5 software (Foss North America, Inc., Eden Prairie, MN). Calibration was done by evaluating spectra for outliers (‘H’ > 3.0) prior to sample selection. The ‘H’ statistic (0.6) was used to select samples with different spectra. Total samples with different spectra for Greenhouse Trial 1 was 20 out of 88 samples and for Greenhouse Trial 2, 10 out of 140 samples were selected. The selected samples were then subsequently analyzed for N concentration using a Leco TruMac CN combustion analyzer. Calibration equations were developed for N using modified PLS regression with four cross validations (Table 3.2). One calibration equation was developed using information from both greenhouse trials, and then applied to all samples to estimate N concentrations.

Statistical analysis

PROC GLM in SAS version 9.4 (SAS Institute, Cary, NC) was used to assess the relationship between plant DM production and N uptake. A probability level of <0.05 was determined to have weakly significant, <0.01 to have moderately significant and <0.001 to have highly significant correlations with plant DM and N uptake. A stepwise selection following PROC REG used 24 measured variables to identify the most important variables to predict plant DM and N uptake: the flush of CO_2, cumulative C mineralization, net N mineralization, SMBC, total soil N, total organic C, particulate organic C and N, inorganic N, available N, bulk density, clay concentration, sand concentration, humic matter, cation exchange capacity, pH, phosphorus, potassium, calcium, magnesium, sulfur, manganese, and zinc.

RESULTS

Effect of temperature in greenhouse trials

Temperature was semi-controlled in greenhouse trials. From stepwise selection following PROC REG in SAS version 9.4 (SAS Institute, Cary, NC), average temperature for the last week of greenhouse growth was a significant variable in the plant DM production and N uptake (Appendix B.2). Average temperature for the last week of Greenhouse Trial 1 was 26˚ C and 22˚ C for Greenhouse Trial 2. Therefore, two greenhouse experiments were analyzed separately.

Relationship between plant dry matter and soil biological properties

Dry matter of sorghum-sudan grass for Greenhouse Trial 1 varied from 4 to 47 mg g^{-1} soil and from 3 to 14 mg g^{-1} soil in Trial 2. Linear regression analysis of plant DM production versus the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC in Greenhouse Trial 1 was highly significant (r^2 = 0.56, 0.63, 0.64 and 0.55 respectively; Table 3.7. and Fig. 3.1). Similarly, linear regression analyses of plant DM production versus soil biological quality measures showed the flush of CO_2, cumulative C mineralization, net N mineralization and soil microbial biomass C being correlated with plant DM production in Greenhouse Trial 2 with coefficient of determination of 0.66, 0.59, 0.62, and 0.58 respectively (Table 3.7 and Fig. 3.1.). Particulate organic N and particulate organic C were not significantly correlated to plant DM in either greenhouse trial (Table 3.7).

We also evaluated total soil N and organic C fractions. Total soil N was significantly correlated to plant DM production for both Trial 1 and Trial 2 ($r^2 = 0.72$ and 0.52 respectively; Table 3.7 and Figure 3.2). For both greenhouse trials, soil organic C was correlated to plant DM production ($r^2 = 0.70$ and 0.37 respectively; Table 3.7).

For both trials, inorganic N had no effect on plant DM production. However, adding inorganic N to net N mineralization improved the correlation for plant DM production versus net N mineralization in Trial 1 from 0.62 to 0.66 (Table 3.7).

Relationship between plant N uptake and soil biological properties

Nitrogen uptake by sorghum-sudangrass varied from 29 to 213 mg kg^{-1} soil in Greenhouse Trial 1 and from 27 to 241 mg kg^{-1} soil in Greenhouse Trial 2. For both trials, plant N uptake was significantly correlated with the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC versus plant N uptake (Table 3.7 and Fig. 3.3). Among the measured active C and N fractions of soils, particulate organic C and N were not significantly correlated to plant N uptake (Table 3.7).

Total soil N was significantly correlated with plant N uptake for both trials (Table 3.7). Similarly, linear regression analysis of soil organic C versus plant N uptake was also significant in both trials (Table 3.7).

The relationship between inorganic N and plant N uptake was also analyzed. There was no significant relationship between the two in both trials. Available N, which was addition of inorganic N to net N mineralization, improved the relationship between plant N uptake and net N mineralization from 0.58 to 0.65 in Greenhouse Trial 1and from 0.68 to 0.70 in Greenhouse Trial 2 (Table 3.7 and Fig. 3.4).

Relationship between soil physical and chemical properties with plant DM and plant N uptake

For Greenhouse Trial 1, some of the variations in the relationship between plant DM and soil biological quality and between plant N uptake and soil biological quality were explained by bulk density, cation exchange capacity, phosphorus, potassium, calcium, magnesium, sulfur, and copper levels. Cation exchange capacity, phosphorus, potassium, calcium, sulfur and copper were strongly positively correlated with plant DM and N uptake. Soil bulk density was negatively correlated to plant DM but not to N uptake. For Greenhouse Trial 2, some of the variations in relationship between plant DM with soil biological quality were explained by cation exchange capacity, potassium, sulfur, calcium and magnesium. These variables were correlated with plant DM and N uptake in a positive manner.

Identifying the key variables to predict plant dry matter and N uptake

Total soil N, cumulative C mineralization, potassium, SMBC, pH, bulk density, sulfur and manganese were selected as key variables at p=0.15 significance level to predict plant DM in Greenhouse Trial 1. A model containing all these variables had an adjusted R^2 of 0.88. pH, and manganese were not significantly correlated with DM in a significance test done using PROC GLM. Therefore, pH, and manganese were removed from the model. Potassium co-varied with soil biological properties in a positive manner (Table3.5) and had a large range from 31 to 759 mg kg^{-1} soil suggesting that potassium might have been limiting macronutrient in some of the soils as the 31 mg potassium kg-1 soil is considered to be low for sorghum-sudan grass ("Fertilization recommendations by crops"). Bulk density had a range from 0.81 to 1.35 g cm^{-3} and it co-varied in a negative direction with soil biological properties (Table 3.5). Soils with higher bulk density had lower value for soil biological properties. For example, Site 15-05 had relatively high bulk density of 1.33 g cm^{-3} and had lower flush of CO_2 (207 mg CO_2-C kg^{-1} soil) than soils with lower bulk density. Sulfur in our soils varied from 9 to 35 mg kg^{-1} soil. 9 mg sulfur kg^{-1} soil is considered to be low for sorghum-sudan grass ("Fertilization recommendations by crops"). Low level of sulfur was seen in Sites 15-03 and 15-06 which might have been limiting to plant growth. Soil samples were taken at 0-10 cm depth from which sulfur might have leached to the subsoil and were limiting for plant growth. Sulfur co-varied with soil biological properties in a positive direction (Table 3.5). Taking these facts into account, potassium, bulk density and sulfur were removed from the model as they were covariates and not necessarily had a direct effect on the plant DM production in Greenhouse Trial 1. Total soil N, cumulative C mineralization and SMBC were considered key variables in the model to predict plant DM in Greenhouse Trial 1 which explained 80% of the variation.

Selected key variables to predict plant N uptake in Greenhouse Trial 1 were the flush of CO_2, inorganic N, total soil N and cumulative C mineralization, which explained 91% of the variation. Inorganic N was not a significant variable when analyzed against N uptake in significance test using PROC GLM (Table 3.7) and thus, was removed from the model. With inorganic N removed, the flush of CO_2, total soil N and cumulative C mineralization explained 82% of the variation.

For Greenhouse Trial 2, the flush of CO_2, cumulative C mineralization, phosphorus, pH, cation exchange capacity, zinc, manganese and clay concentration were selected as the key variables to predict plant DM and N uptake which explained 86% and 99 % of the variation for plant DM and N uptake respectively. Phosphorus, pH, cation exchange capacity, zinc, manganese, and clay concentration were not significantly correlated to plant DM and N uptake in Greenhouse Trial 2 (Table 3.7) and thus were removed from the model. The model with cumulative C mineralization, and the flush of CO_2 explained 79% and 99% variation in plant DM and N uptake respectively.

Principal component analysis

Since, the selected key variables may have co-varied with each other, the effect of these variables independent of covariance was analyzed by principal component analysis.

For DM production in Greenhouse Trial 1, the first principal component explained 86%, the second principal component explained 10%, and the third principal component explained <1% of the variation (Table 3.4; Figure 3.7). Since the Eigen vectors of three key variables were similar (Table 3.3), all three variables -Cumulative C mineralization, SMBC and total soil N were considered to be equally important.

For N uptake in Greenhouse Trial 1, the first principal component explained 87% and the second and third principal components explained <1% each (Table 3.4; Figure 3.8). The Since the value of eigenvectors were similar for the flush of CO_2, total N and cumulative C mineralization (Table 3.3), all three variables were equally important in predicting plant N uptake.

Key variables selected to predict plant DM and N uptake in Greenhouse Trial 2 were the flush of CO_2 and cumulative C mineralization. First principal component explained 99% of the variation with high Eigen value while second principal component explained <1% of the variation in plant DM and N uptake (Table 3.4; Figure 3.9). Eigen vectors for both the flush of CO_2 and cumulative C mineralization were similar suggesting equal importance of both the variables in predicting plant DM and N uptake in Greenhouse Trial 2.

Relationship between net N mineralization and other soil biological properties

The flush of CO_2 was strongly correlated to net N mineralization for Greenhouse Trial 1 ($r^2 = 0.61$) and Greenhouse Trial 2 ($r^2 = 0.56$) (Fig. 3.6). Cumulative C mineralization, SMBC, total soil N, and soil organic C were also strongly correlated with net N mineralization in Greenhouse Trial 1 ($r^2 = 0.42$, 0.57, 0.68 and 0.66, respectively). Cumulative C mineralization, SMBC, total soil N and soil organic C were also strongly correlated with net N mineralization in Greenhouse Trial 2 ($r^2 = 0.54$, 0.49, 0.64 and 0.55, respectively). Particulate organic N and C had weak correlation with net N mineralization in both trials ($r^2 = 0.17$ and 0.20 respectively; Figure not shown).

DISCUSSION

The findings from this study suggest that plant DM production and plant N uptake in a semi-controlled environment relates to the flush of CO_2, cumulative C mineralization, net N mineralization, and SMBC. Our hypothesis that active soil C and N fractions relates to plant DM production and N uptake was accepted. Previous studies on indices for predicting N mineralization (Schomberg et al., 2009), and the relationship between the flush of CO_2 and crop N uptake (Haney et al., 2001) have laid the foundation of this study. The findings of this research verified the findings of Pershing (2016).

Among the active soil C and N fractions measured in the study, particulate organic C and N were not correlated with plant DM and N uptake. This result contradicts the result in similar semi-controlled greenhouse experiments where there was a good correlation between yield and N uptake with particulate organic C and N fractions (Pershing, 2016). This finding also contradicts the field correlation of particulate organic C with yield (Majumder et al., 2007). In contrast, Schomberg et al. (2009) found that particulate organic C and N were not as useful in predicting mineralizable N. Particulate organic C and N in our study were also weakly correlated with net N mineralization, questioning their ability to predict N availability in the soil. It seems that particulate organic C and N were not as mineralizable a source of N during 6-week greenhouse study.

Total soil N and soil organic C correlated to plant DM and N uptake in both trials. Total soil N and soil organic C being strongly correlated to plant DM and N uptake suggest that soil C and N pools were active rather than recalcitrant. There was no evidence of initial inorganic N and humic matter being correlated to plant DM production and N uptake.

Among the active soil C and N fractions, cumulative C mineralization, SMBC and total soil N were key variables to predict plant DM in Greenhouse Trial 1. Similarly, cumulative C mineralization, the flush of CO_2 and total soil N were key variables to predict plant N uptake in Greenhouse Trial 1 and cumulative C mineralization and the flush of CO_2 were key variables in predicting plant DM and N uptake in Greenhouse Trial 2. On average, the flush of CO_2 explained the greatest amount of variation in plant DM and N uptake in both trials. Similar results were found in greenhouse trials conducted with diverse soils from North Carolina and Virginia ($r^2 = 0.66$ for plant DM and 0.81 for plant N uptake; Pershing, 2016). The flush of CO_2 had strong correlation with net N mineralization which is similar to the findings of Franzluebbers and Brock (2007) and Franzluebbers and Stuedemann (2008). The fact that the flush of CO_2 takes only 3 days to measure might have helped to decrease experimental error compared with other soil biological quality measures. The flush of CO_2 being rapid, reproducible, easy to measure, accessible to many users and having the capacity to integrate physical, chemical and biological soil properties, has potential to be used as a soil health tool (Franzluebbers, 2016).

A limitation of this study is the lack of complete data set for temperature during the greenhouse growth. Although last week average temperature was significantly different for two greenhouse trials, the effect of overall temperature on plant growth is not available.

Current soil testing services focus on total soil organic matter, chemical soil indicators of inorganic N, P, K, soil pH and various other macro and micro nutrients to assess nutrient availability to crops (Franzluebbers, 2016). Although soil biological quality is not routinely tested, estimating soil N mineralization potential is important for maximizing N-use efficiency and minimizing environmental losses (Schomberg et al. 2009). Therefore, some estimation of soil biological quality would be appropriate for routine soil testing.

CONCLUSION

Greenhouse trials using tall fescue pasture soils revealed a strong positive correlation of the flush of CO_2, cumulative C mineralization, net N mineralization and SMBC with plant DM and N uptake. Particulate organic C and N were not correlated to plant DM and N uptake. Greenhouse trials confirm that N taken up by bioassay plants are due to mineralizable N from active soil N fractions. These data clearly suggest that soil biological activity should be a routine soil testing tool for evaluating N availability in the field.

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Table 3.1. Water supply and monitoring in greenhouse trials.

	Greenhouse Trial 1			Greenhouse Trial 2		
Days in greenhouse	[1]Before watering (g)	[2]After watering (g)	[3]Water uptake (g)	Before watering (g)	After watering (g)	Water uptake(g)
1	8170	-	-	9850	-	-
2	-	-	-	-	-	-
3	-	-	-	9440	-	-
4	7810	-	-	9260	-	-
5	7690	-	-	8990	9610	620
6	7330	7800	470	9530	-	-
7	-	-	-	-	9980	-
8	6930	7580	650	-	-	-
9	-	-	-	-	-	-
10	6860	7440	580	8730	9460	730
11	7320	8050	730	8800	9400	600
12	7670	-	-	9100	9500	400
13	7290	7720	430	8980	-	-
14	7160	7690	530	8780	9770	990
15	7200	7640	440	-	-	-
16	7170	7580	410	8470	9190	720
17	7020	7490	470	8890	9400	510
18	7030	8160	1130	8730	9350	620
19	-	-	-	8990	9420	430
20	7210	7690	480	8990	9450	460
21	7230	7690	460	8810	9100	290
22	7370	7690	320	-	9210	-
23	7320	7660	340	8870	9450	580

Table 3.1. Continued

24	7200	7650	450	9360	-	-
25	7230	8210	980	8710	9370	660
26	-	-	-	-	-	-
27	7230	7680	450	8660	9240	580
28	7390	7760	370	8780	9600	820
29	7300	7730	430	9250	-	-
30	7210	7690	480	-	-	-
31	7200	7640	440	8510	9130	620
32	7390	8330	940	8790	9320	530
33	7520	-	-	8720	9270	550
34	7310	7720	410	8750	9230	480
35	7380	7780	400	8820	9120	300
36	7530	7760	230	-	-	-
37	-	-	-	-	-	-
38	7170	7610	440	9070	-	-
39	-	-	-	8600	-	-
40	7190	-	-	-	-	-
41	-	-	-	-	-	-
42	-	-	-	-	-	-

[1] Weight of a tray before watering.

[2] Weight of the tray after watering.

[3] Net weight of water taken up by the tray.

Table 3.2. Near infrared spectroscopy calibration for equation development for greenhouse trials.

Greenhouse trial number	Variable	No. of samples used for calibration (N)	Mean of N	Standard error of calibration (SEC)	SEC R-square	Standard error of cross validation (SECV)	SECV R-square	SEC as % OF Mean	SECV as % OF Mean	Math Treatment
1	Nitrogen	224	1.112	0.02	0.994	0.032	0.990	2.094	2.886	1,4,4,1
2	Nitrogen	254	1.106	0.03	0.991	0.037	0.987	1.454	1.783	1,4,4,4

Table 3.3. Eigen vectors of each key variables to predict plant dry matter and nitrogen uptake in principal component analysis in Greenhouse Trail 1 and 2.

Greenhouse trial number	Response variable	Key predictors	Principal component 1	Principal component 2	Principal component 3
1	Plant DM	Cumulative C mineralization	0.56	0.71	0.42
		Soil microbial biomass C	0.60	0.00	-0.80
		Total soil N	0.56	-0.71	0.43
1	Plant N uptake	Cumulative C mineralization	0.59	-0.50	0.64
		Flush of CO_2	0.60	-0.26	-0.75
		Total soil N	0.54	0.83	0.14
2	Plant DM	Flush of CO_2	0.71	0.71	-
		Cumulative C mineralization	0.71	-0.71	-
3	N uptake	Flush of CO_2	0.71	0.71	-
		Cumulative C mineralization	0.71	-0.71	-

Table 3.4. Eigen value and proportion of each principal components to predict plant dry matter and nitrogen uptake in Greenhouse Trail 1 and Trial 2.

Greenhouse trial number	Response variable	Principal components	Eigenvalue	Difference	Proportion	Cumulative
1	Plant DM	1	2.59	2.28	0.86	0.86
		2	0.31	0.22	0.10	0.97
		3	0.09		0.03	1.00
1	Plant N uptake	1	2.60	2.26	0.87	0.87
		2	0.34	0.29	0.11	0.98
		3	0.05		0.02	1.00
2	Plant DM/N uptake	1	1.99	1.99	1.00	1.00
		2	0.00		0.00	1.00

Table 3.5. Correlation matrix among all the independent variables of Greenhouse Trial 1.

Variables	1	2	3	4	5	6	7
1. Flush of CO_2	-						
2. Cumulative C mineralization	0.94^{***}	-					
3. Net N mineralization	0.78^{***}	0.65^{***}	-				
4. Soil microbial biomass C	0.88^{***}	0.85^{***}	0.76^{***}	-			
5. Total soil N	0.77^{***}	0.69^{***}	0.83^{***}	0.85^{***}	-		
6. Total organic C	0.72^{***}	0.64^{***}	0.82^{***}	0.80^{***}	0.97^{***}	-	
7. Particulate organic N	0.36^{***}	0.33^{***}	0.46^{***}	0.39^{***}	0.36^{***}	0.38^{***}	-
Mean	346.47	966.32	111.12	1286.00	2.23	26.35	0.20
Standard Deviation (SD)	125.12	330.98	56.00	424.29	0.93	10.30	0.07

Table 3.5. Continued

Variables	1	2	3	4	5	6	7
8. Particulate organic C	0.37**	0.36***	0.41***	0.39***	0.40***	0.40***	0.90***
9. Bulk density	-0.57***	-0.58***	-0.56***	-0.63***	-0.62***	-0.57***	-0.22***
10. Clay concentration	0.23*	0.16	0.31**	0.32**	0.40***	0.36**	0.09
11. Inorganic N	0.56***	0.48***	0.58***	0.54***	0.62***	0.57***	0.38***
12. Available N	0.79***	0.66***	0.99***	0.77***	0.84***	0.82***	0.47***
13. Humic matter	0.18	0.10	0.43***	0.20	0.56***	0.67***	0.07
14. Cation exchange capacity	0.66***	0.67***	0.53***	0.72***	0.70***	0.69***	0.49***
15. pH	-0.16	-0.15	-0.19	-0.14	-0.23	-0.20	0.19
16. Phosphorus	0.12	0.00	0.32***	0.18	0.43***	0.43***	0.20
17. Potassium	0.64***	0.51***	0.72***	0.61***	0.72***	0.70***	0.39***
18. Calcium	0.46***	0.46***	0.34***	0.52***	0.51***	0.49***	0.40***
19. Magnesium	0.51***	0.62***	0.35***	0.57***	0.39***	0.40***	0.44***
20. Sulfur	0.62***	0.56***	0.65***	0.62***	0.75***	0.73***	0.27*
21. Manganese	0.28*	0.28*	0.21	0.22*	0.22*	0.11	-0.06
22. Zinc	0.32***	0.30***	0.42***	0.29*	0.20	0.30***	0.35***
23. Copper	0.37***	0.37***	0.32**	0.40***	0.40**	0.42***	0.16

Table 3.5. Continued

Variables	8	9	10	11	12	13	14	15	16	17	18
8	-										
9	-0.17	-									
10	0.03	-0.15	-								
11	0.31***	-0.36***	0.19	-							
12	0.42***	-0.56***	0.31***	0.70***	-						
13	0.15	-0.27*	0.25*	0.19	0.41***	-					
14	0.44***	-0.45***	0.27*	0.53***	0.57***	0.17	-				
15	0.14	0.22*	-0.27*	-0.01	-0.17	-0.34***	0.33***	-			
16	0.13	-0.07	0.43***	0.60***	0.39***	0.34***	0.31***	0.01	-		
17	0.34***	-0.34***	0.28*	0.87***	0.80***	0.35***	0.58***	-0.01	0.65***	-	
18	0.32***	-0.27*	0.15	0.45***	0.38***	0.01	0.92***	0.56***	0.32***	0.48***	-
Mean	4.18	1.15	0.20	14.43	125.55	5.85	10.74	6.08	91.69	163.73	1320.00
SD	1.31	0.17	0.08	11.80	63.62	6.02	3.88	0.48	49.61	143.12	619.39

Table 3.5. Continued

Variables	19	20	21	22	23
19	-				
20	0.33^{***}	-			
21	0.22^{*}	0.51^{***}	-		
22	0.47^{***}	0.33^{***}	0.02	-	
23	0.17	0.52^{***}	0.31^{***}	0.23^{*}	-
Mean	287.07	20.30	67.93	8.44	2.57
SD	134.22	7.10	64.64	5.63	1.60

*significant at 0.05 probability level

**significant at 0.01 probability level

***significant at 0.001 probability level
NS not significant

Table 3.6**.** Correlation matrix among all the response and independent variables of Greenhouse Trial 2.

Variables	1	2	3	4	5	6	7
1. Flush of CO_2	-						
2. Cumulative C mineralization	0.99^{***}	-					
3. Net N mineralization	0.75^{***}	0.79^{***}	-				
4. Soil microbial biomass C	0.68^{***}	0.69^{***}	0.71^{***}	-			
5. Total soil N	0.76^{***}	0.79^{***}	0.80^{***}	0.82^{***}	-		
6. Total organic C	0.69^{***}	0.71^{***}	0.75^{***}	0.78^{***}	0.94^{***}	-	
7. Particulate organic N	0.24^{*}	0.23^{*}	0.30^{***}	0.44^{***}	0.40^{***}	0.35^{***}	-
Mean	380.31	459.65	125.57	1340	2.24	26.66	0.24
Standard Deviation (SD)	134.53	166.58	56.80	372.93	0.80	9.17	0.10

Table 3.6. Continued

Variables	3	4	5	6	7	8	9	10	11
8. Particulate organic C	0.12	0.17*	0.30***	0.28***	0.30***	0.49***	0.39***	0.45***	0.79***
9. Bulk density	-0.11	-0.15	-0.18*	-0.18*	-0.14	-0.20*	-0.17	-0.12	-0.24***
10. Clay concentration	0.18*	0.09	0.07	0.08	0.07	0.05	0.13	0.10	0.27***
11. Inorganic N	0.13	0.10	0.10	0.10	0.04	0.00	0.08	0.05	0.20*
12. Available N	0.35***	0.42***	0.45***	0.46***	0.39***	0.36***	0.38***	0.35***	0.08
13. Humic matter	0.04	0.07	0.02	0.03	0.08	0.07	0.19*	0.42***	-0.04
14. Cation exchange capacity	0.32***	0.29***	0.32***	0.32***	0.38***	0.55***	0.53***	0.45***	0.35***
15. pH	0.17	0.07	0.04	0.04	0.06	0.04	0.12	0.09	0.27***
16. Phosphorus	0.09	-0.13	-0.22*	-0.20*	-0.04	0.06	0.06	0.01	0.13
17. Potassium	0.32***	0.23*	0.17	0.19*	0.26***	0.25***	0.30***	0.22*	0.44***
18. Calcium	0.23*	0.17*	0.22*	0.22*	0.23*	0.44***	0.42***	0.35***	0.27***
19. Magnesium	0.19*	0.17*	0.15	0.16	0.36***	0.38***	0.26***	0.19*	0.32***
20. Sufur	0.53***	0.56***	0.53***	0.55***	0.50***	0.37***	0.48***	0.43***	0.21*
21. Manganese	0.16	0.06	0.14	0.13	0.08	0.05	0.01	-0.09	-0.19*
22. Zinc	-0.02	-0.09	-0.14	-0.14	0.11	0.17	0.08	0.07	0.23*
23. Copper	0.16	0.03	0.03	0.03	0.07	0.16	0.15	0.13	0.20*

Table 3.6. Continued

Variables	8	9	10	11	12	13	14	15	16	17	18
8	-										
9	-0.22*	-									
10	-0.20*	-0.07	-								
11	-0.24***	-0.07	0.81***	-							
12	0.19*	-0.13	-0.15	-0.16	-						
13	0.03	0.20*	0.15	0.16	-0.02	-					
14	0.34***	-0.11	-0.10	-0.13	0.20*	-0.01	-				
15	-0.19*	-0.07	0.99***	0.73***	-0.14	0.14	-0.08	-			
16	0.00	0.04	0.07	0.01	-0.08	-0.05	0.42***	0.10	-		
17	-0.03	-0.12	0.79***	0.78***	-0.08	0.06	0.25***	0.76***	0.30***	-	
18	0.28***	-0.09	-0.12	-0.16	0.15	-0.02	0.97***	-0.09	0.42***	0.18*	-
Mean	4.35	1.48	0.48	24.28	300.66	5.19	11.28	7.14	108.93	183.34	1491
SD	1.67	1.92	2.56	74.31	206.6	5.58	4.75	10.06	99.81	180.42	846.95

Table 3.6. Continued

Variables	19	20	21	22	23
19	-				
20	-0.02	-			
21	0.05	0.03	-		
22	0.63***	-0.02	-0.06	-	
23	0.06	0.09	0.24***	0.27***	-
Mean	245.38	18.78	68.77	10.71	3.62
SD	130.07	6.69	57.65	8.14	3.70

*significant at 0.05 probability level

**significant at 0.01 probability level

***significant at 0.001 probability level
NS not significant

Table 3.7. Coefficients of determination from linear regression models of soil properties versus plant DM and N uptake for Greenhouse Trial 1 and Trial 2.

Soil property	Plant DM production		Plant N uptake	
	Greenhouse Trial 1	Greenhouse Trial 2	Greenhouse Trial 1	Greenhouse Trial 2
Flush of CO_2	0.66^{***}	0.56^{***}	0.77^{***}	0.72^{***}
C mineralization	0.59^{***}	0.63^{***}	0.72^{***}	0.81^{***}
N mineralization	0.62^{***}	0.64^{***}	0.58^{***}	0.68^{***}
Soil microbial biomass C	0.58^{***}	0.55^{***}	0.70^{***}	0.37^{***}
Particulate organic N	NS	NS	NS	NS
Particulate organic C	NS	NS	NS	NS
Total N	0.72^{***}	0.52^{***}	0.72^{***}	0.62^{***}
Total C	0.70***	0.37***	0.60***	0.48***
Inorganic N	NS	NS	NS	NS
Available N	0.66^{***}	0.56^{***}	0.65^{***}	0.70^{***}
Bulk density	0.33^{***}	NS	0.34^{***}	NS
Humic matter	NS	NS	NS	NS
CEC	0.55^{***}	0.49^{***}	0.12^{*}	NS
pH	NS	NS	NS	NS
Phosphorus	0.17^{*}	NS	0.14^{*}	NS
Potassium	0.60^{***}	0.63^{***}	0.10^{*}	0.06^{*}
Calcium	0.35^{***}	0.06^{**}	0.26^{**}	0.06^{*}
Magnesium	0.17^{*}	0.06^{*}	0.17^{*}	0.04^{*}
Sulfur	0.54^{***}	0.53^{***}	0.49^{***}	0.52^{***}
Manganese	NS	NS	NS	NS
Zinc	NS	NS	NS	NS
Copper	0.47^{***}	0.24^{*}	NS	NS

*significant at 0.05 probability level

**significant at 0.01 probability level

***significant at 0.001 probability level
NS not significant

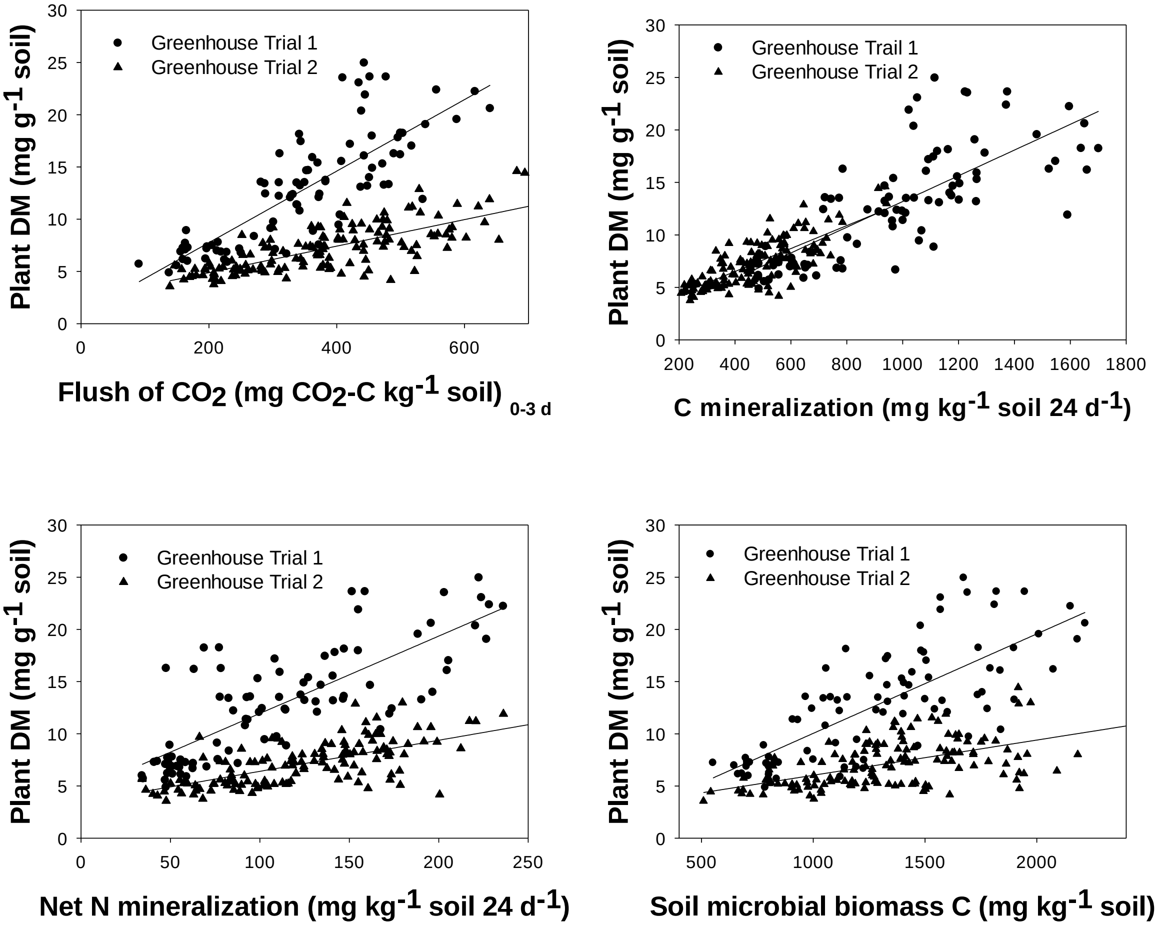


Figure 3.1.Sorghum-sudan dry matter as a function of active soil biological quality measures for Greenhouse Trial 1 and Trial 2.

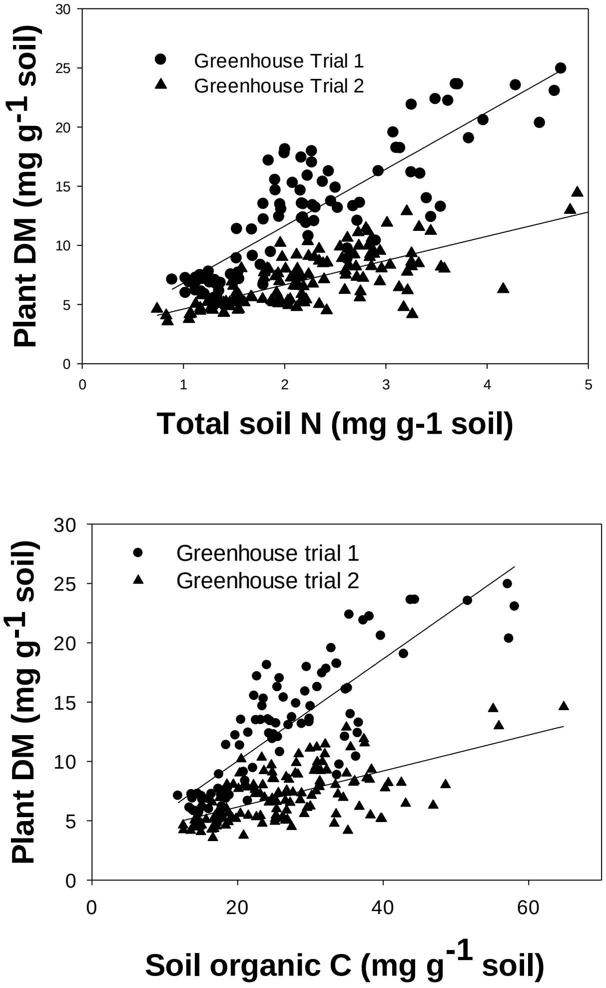


Figure 3.2.Sorghum-sudan dry matter as a function of total soil N and soil organic C for Greenhouse Trial 1 and Trial 2.

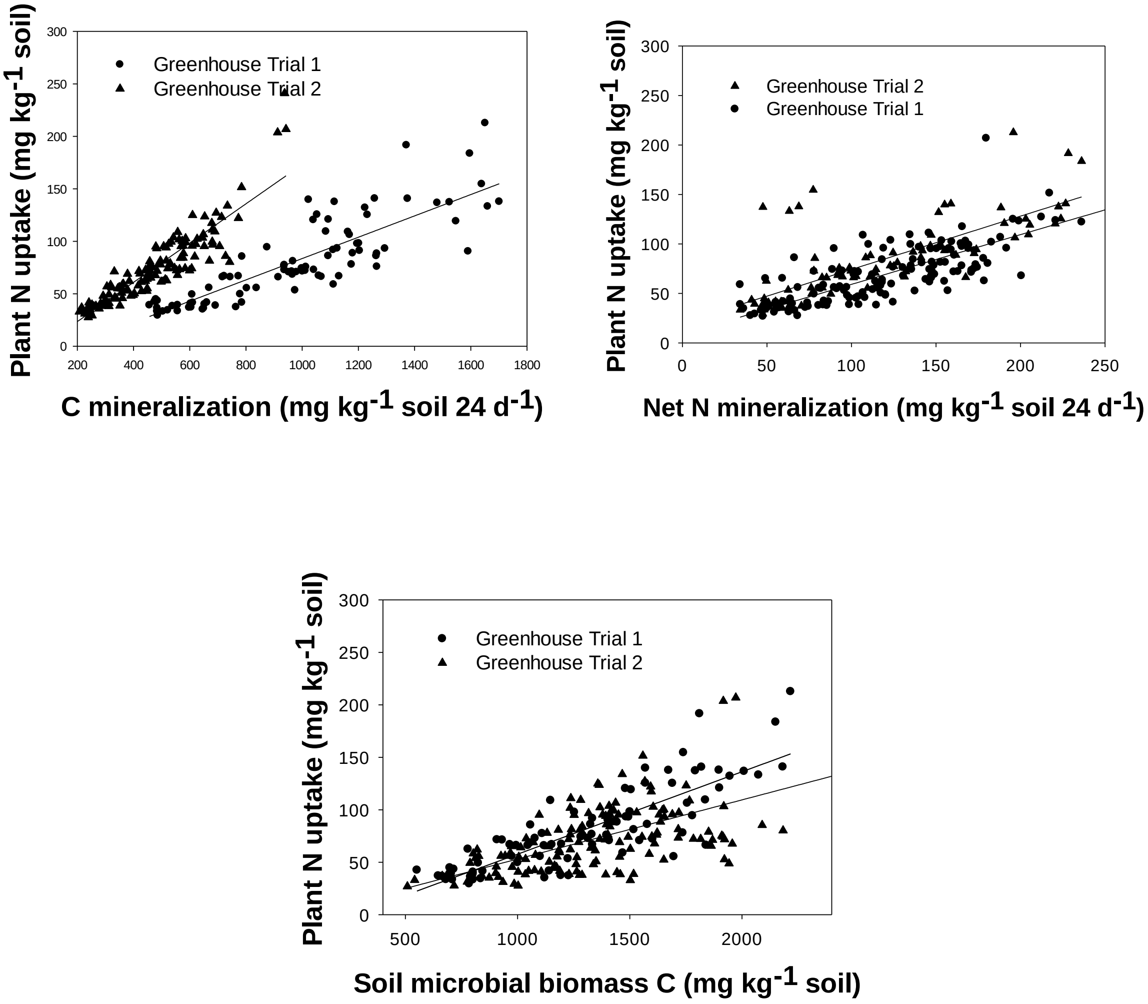


Figure 3.3.Sorghum-sudan N uptake as a function of cumulative carbon mineralization, net nitrogen mineralization and soil microbial biomass carbon for Greenhouse Trial 1 and Trial 2.

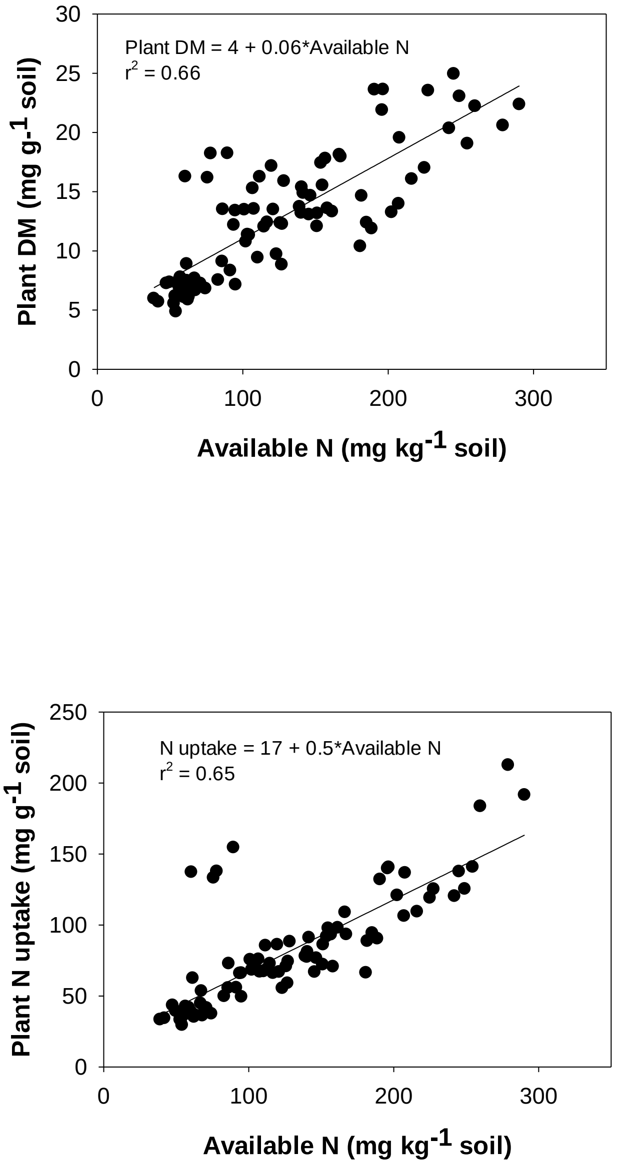
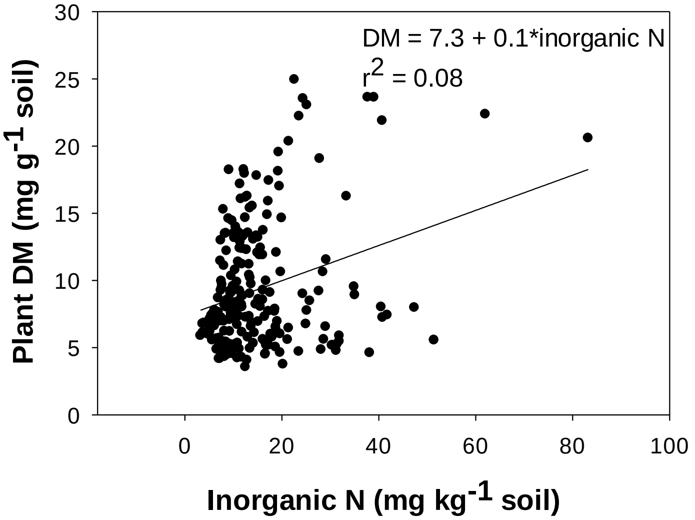


Figure 3.4. Sorghum-sudan DM and N uptake as a function of available nitrogen (Greenhouse Trial 1).



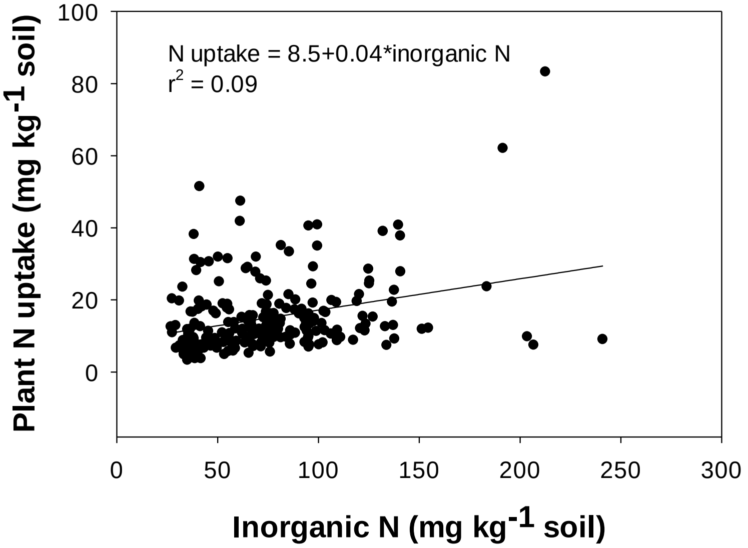


Figure 3.5. Sorghum-sudan DM and N uptake as a function of inorganic nitrogen (greenhouse trial 1)

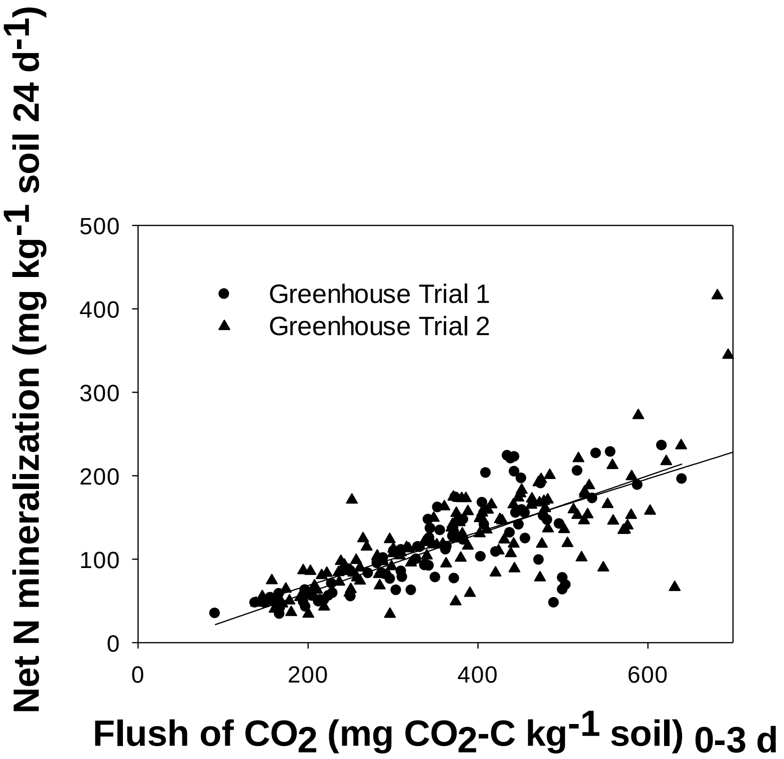


Figure 3.6. Relationship between the flush of CO_2 and net N mineralization (Greenhouse Trial 1 and Greenhouse Trial 2).

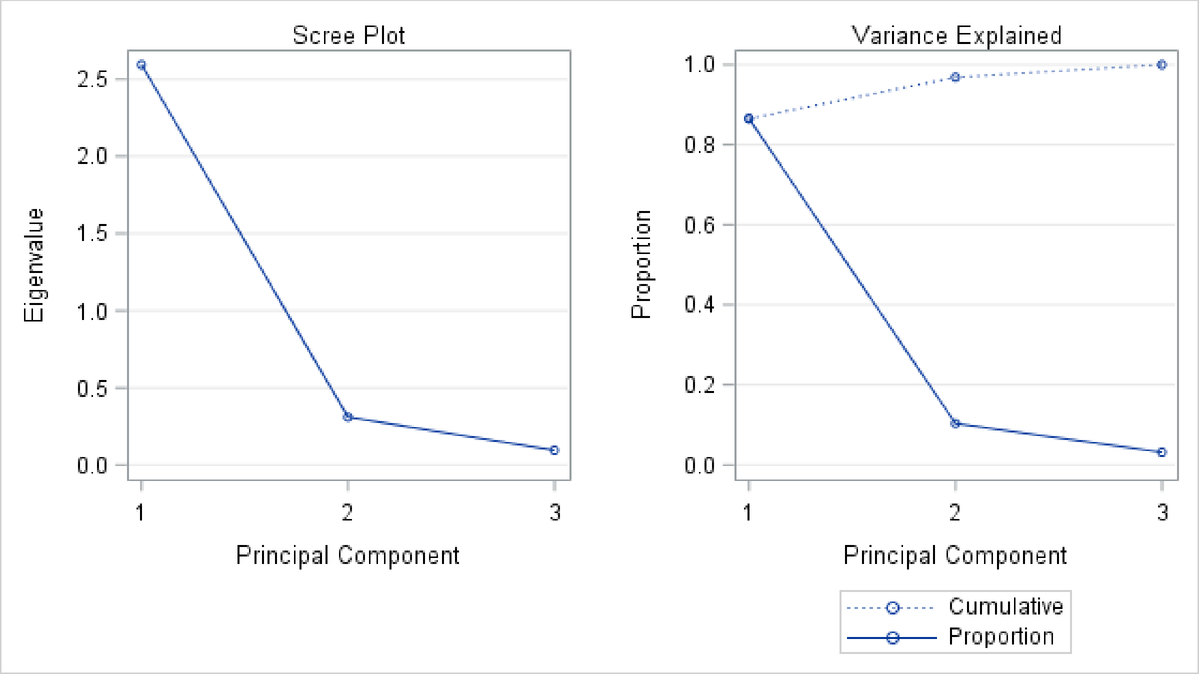


Figure 3.7. Principal component analysis of plant dry matter in Greenhouse Trial 1 using total soil nitrogen, cumulative C mineralization and soil microbial biomass C (key variables selected by stepwise procedure).

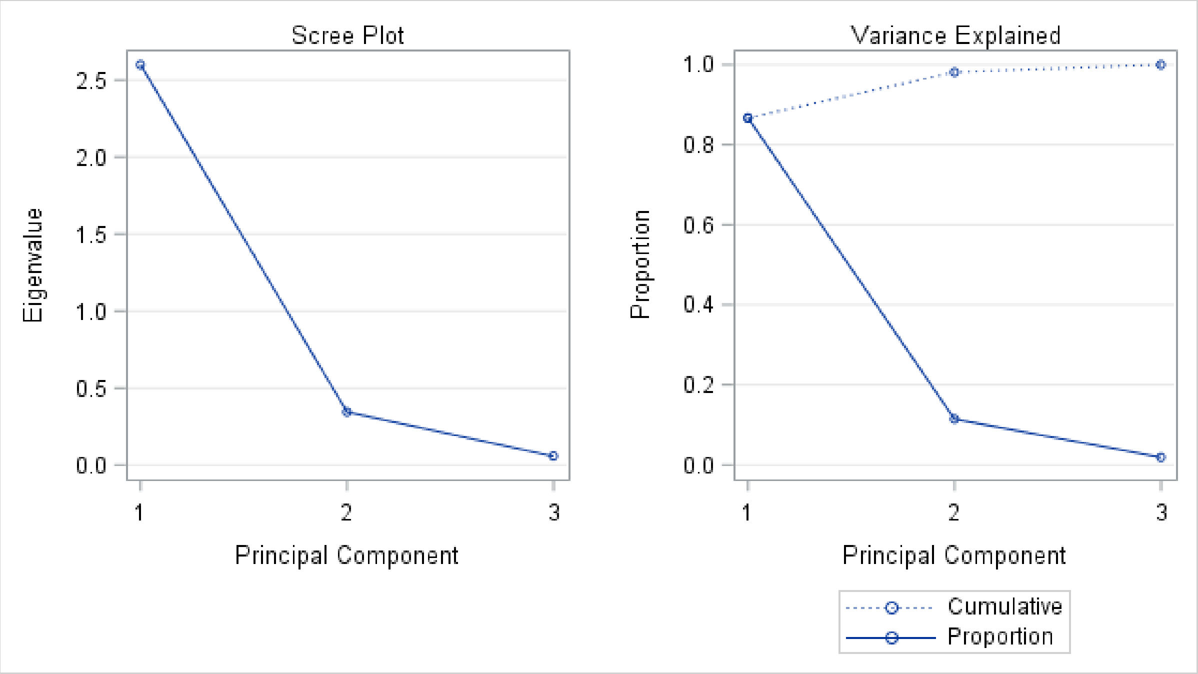


Figure 3.8. Principal component analysis of plant nitrogen uptake in Greenhouse Trial 1 using total soil nitrogen, the flush of CO_2, and cumulative C mineralization (key variables selected by stepwise procedure).

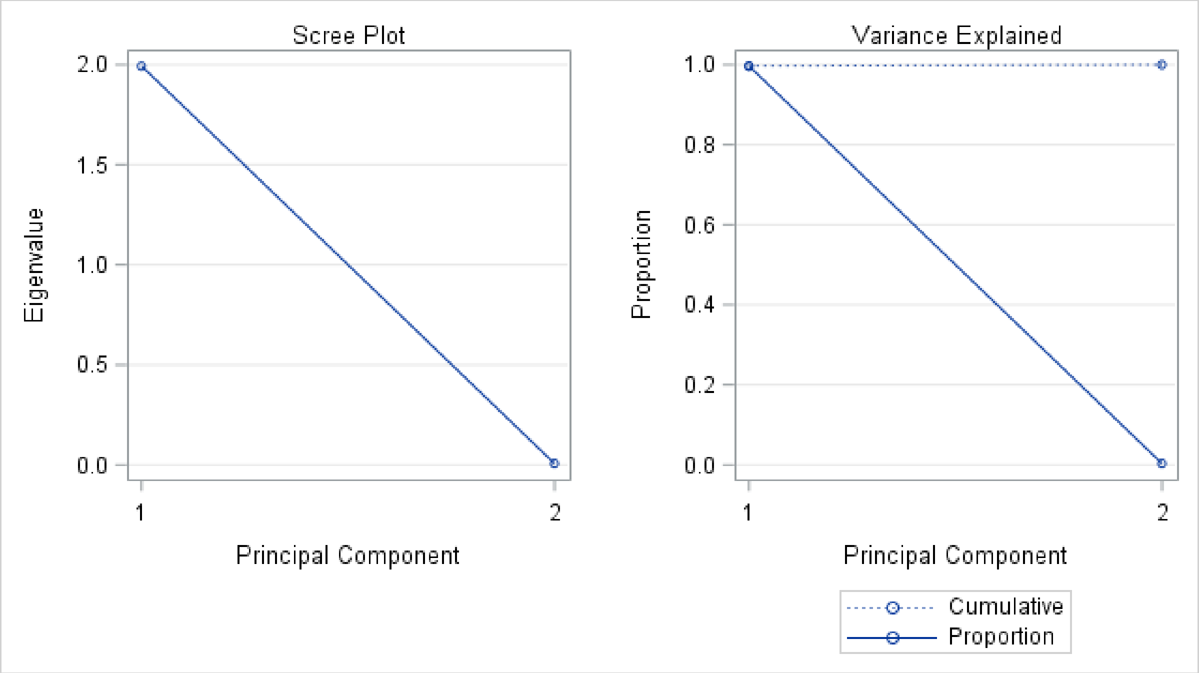


Figure 3.9. Principal component analysis of plant dry matter/nitrogen uptake in Greenhouse Trial 2 using cumulative C mineralization and the flush of CO_2 (key variables selected by stepwise procedure).

APPENDICES

Appendix A: Nitrogen rate effect on dry matter, crude protein and moisture for individual sites along with soil properties

Site 15-01; Lake Wheeler Road Research Station, Wake County, NC

- Cecil gravelly sandy loam, 6 to 10% slopes, moderately eroded (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+ fescue, >10 years pasture
- Soil sampled 26 Aug, fertilized 2 Sep 2015, harvested 19 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	1646^{ba}	29.2^{c}	11.1^{c}	55.5^{a}
45	1768^{ba}	34.4^{b}	12.1^{b}	55.5^{a}
90	1582^{b}	34.5^{cb}	13.6^{a}	54.7^{a}
135	1867^{a}	42.3^{a}	14.1^{a}	55.8^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1152	20.6
Residue	1244	14.7

Property	**Mean**	**CV (%)**
Apparent nitrification (%)	84	2
——— g kg^{-1} ———		
Total C	34.6	3
Total N	2.71	5
Particulate organic C	5.26	21
Particulate organic N	0.22	27
——— mg kg^{-1} ———		
Inorganic N	14	2
Soil microbial biomass C	1651	9
C mineralization (24 d^{-1})	998	13
N mineralization (24 d^{-1})	131	20
Flush of CO_2 (3 d^{-1})	360	12

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.08	10
Humic matter (g kg^{-1})	7.3	1
CEC (cmol$_c$ kg^{1})	9.8	32
pH	5.8	25
——— mg kg^{-1} ———		
P	58	9
K	77	9
Ca	1069	5
Mg	318	7
S	20	8
Mn	21	7
Zn	8	0
Cu	1	5
Na	0	3

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	16	13	13	4
Precipitation (mm)	145	133	180	161	38

Site 15-02; Central Crops Research Station, Johnston County, NC

- Wedowee sandy loam, 2 to 8 % slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Mowed, E+ fescue, >20 years pasture
- Soil sampled 19 Aug, fertilized 2 Sep 2015, harvested 18 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	1491^{a}	27.6^{b}	11.5^{b}	62.8^{b}
45	1680^{a}	35.6^{a}	13.2^{a}	66.1^{a}
90	1572^{a}	36.0^{a}	14.4^{a}	65.4^{ba}
135	1717^{a}	39.5^{a}	14.5^{a}	64.8^{ba}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1086	19.2
Residue	1881	13.4

Property	Mean	CV (%)	Property	Mean	CV (%)
Apparent nitrification (%)	74	18	Bulk density (Mg m^{-3})	1.23	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.3	35
Total C	20.2	7	CEC (cmol$_c$ kg^{1})	6.4	6
Total N	1.62	8	pH	5.6	2
Particulate organic C	2.67	12	——— mg kg^{-1} ———		
Particulate organic N	0.11	24	P	38	8
——— mg kg^{-1} ———			K	115	17
Inorganic N	8	17	Ca	689	17
Soil microbial biomass C	974	11	Mg	154	8
C mineralization (24 d^{-1})	723	14	S	15	5
N mineralization (24 d^{-1})	81	7	Mn	17	10
Flush of CO_2 (3 d^{-1})	297	18	Zn	4	11
			Cu	1	5
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	16	13	13	4
Precipitation (mm)	128	115	195	177	39

Site 15-03; Cherry Research Farm, Wayne County, NC

- Johns sandy loam (fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic, Aquic Hapludults)
- Hayed, Novel E+ , 1 year pasture
- Soil sampled 18 Aug, fertilized 2 Sep 2015, harvested 18 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	1370^{a}	21.5^{c}	9.72^{c}	61.8^{b}
45	1521^{a}	26.6^{bc}	10.68^{c}	63.9^{b}
90	1613^{a}	32.3^{ba}	12.15^{b}	65.9^{ba}
135	1498^{a}	35.7^{a}	14.04^{a}	68.3^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1326	22.3
Residue	1612	10.1

Property	Mean	CV (%)	Property	Mean	CV (%)
Apparent nitrification (%)	82	9	Bulk density (Mg m^{-3})	1.29	1
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3	31
Total C	14.8	8	CEC (cmol$_c$ kg^{1})	8	15
Total N	1.27	7	pH	6.3	4
Particulate organic C	2.67	20	——— mg kg^{-1} ———		
Particulate organic N	0.13	25	P	32	11
——— mg kg^{-1} ———			K	47	2
Inorganic N	4	8	Ca	981	18
Soil microbial biomass C	1064	17	Mg	243	24
C mineralization (24 d^{-1})	722	9	S	10	7
N mineralization (24 d^{-1})	60	12	Mn	60	8
Flush of CO$_2$ (3 d^{-1})	233	5	Zn	8	37
			Cu	3	7
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	16	13	12	4
Precipitation (mm)	111	166	220	85	64

Site 15-04; LB-East field, Pender County, NC

- Exum loam, 0 to 2 percent slopes (Fine-silty, siliceous, subactive, thermic Aquic Paleudults); Aycock loam, 0 to 3 percent slopes (Fine-silty, siliceous, subactive, thermic Typic Paleudults)
- Rotationally grazed, Novel E+ fescue, 5 years pasture
- Soil sampled 22 Aug, fertilized 2 Sep 2015, harvested 18 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	NA	NA	NA	NA
45	NA	NA	NA	NA
90	NA	NA	NA	NA
135	NA	NA	NA	NA

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1414	13.9
Residue	2450	14.3

Property	**Mean**	**CV (%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	87	10	Bulk density (Mg m^{-3})	1.36	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	6.8	6
Total C	17.1	6	CEC (cmol$_c$ kg^{1})	6.5	7
Total N	1.11	6	pH	6.1	1
Particulate organic C	4.63	10	——— mg kg^{-1} ———		
Particulate organic N	0.17	20	P	90	6
——— mg kg^{-1} ———			K	77	15
Inorganic N	6	20	Ca	915	10
Soil microbial biomass C	861	34	Mg	94	6
C mineralization (24 d^{-1})	561	10	S	13	7
N mineralization (24 d^{-1})	47	19	Mn	10	13
Flush of CO_2 (3 d^{-1})	195	10	Zn	6	11
			Cu	3.2	3
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	15	15	5
Precipitation (mm)	90	192	380	168	33

Site 15-05; LB-West field, Pender County, NC

- Exum loam, 0 to 2 percent slopes (Fine-silty, siliceous, subactive, thermic Aquic Paleudults); Aycock loam, 0 to 3 percent slopes (Fine-silty, siliceous, subactive, thermic Typic Paleudults)
- Rotationally grazed, Novel E+ fescue, 5 years pasture
- Soil sampled 22 Aug, fertilized 2 Sep 2015, harvested 18 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	NA	NA	NA	NA
45	NA	NA	NA	NA
90	NA	NA	NA	NA
135	NA	NA	NA	NA

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1436	18.3
Residue	3556	13.0

Property	Mean	CV (%)	Property	Mean	CV (%)
Apparent nitrification (%)	79	12	Bulk density (Mg m^{-3})	1.33	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	6.3	20
Total C	18	2	CEC ($cmol_c$ kg^{1})	7	13
Total N	1.22	3	pH	6.1	3
Particulate organic C	4.18	28	——— mg kg^{-1} ———		
Particulate organic N	0.17	40	P	118	21
——— mg kg^{-1} ———			K	114	19
Inorganic N	6	11	Ca	956	20
Soil microbial biomass C	744	6	Mg	117	12
C mineralization (24 d^{-1})	615	7	S	13	7
N mineralization (24 d^{-1})	52	16	Mn	10	14
Flush of CO_2 (3 d^{-1})	207	6	Zn	8	25
			Cu	4	29
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	15	15	5
Precipitation (mm)	90	192	380	168	33

Site 15-06; Sandhills Research Station, Montgomery County, NC

- Candor sand, 0 to 8% slopes (sandy, kaolinitic, thermic Grossarenic Kandiudults)
- Mowed, E+ fescue; mixed, >10 years pasture
- Soil sampled 19 Aug, fertilized 3 Sep 2015, harvested 12 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	472^{a}	9.7^{b}	12.9^{c}	61.6^{ba}
45	583^{a}	14.1^{a}	15.0^{b}	59.8^{b}
90	614^{a}	16.3^{a}	16.6^{a}	64.6^{a}
135	622.2^{a}	17.1^{a}	17.2^{a}	64.5^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	796	30.1
Residue	3044	19.7

Property	Mean	CV (%)	Property	Mean	CV (%)
Apparent nitrification (%)	68	31	Bulk density (Mg m^{-3})	1.27	1
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.5	32
Total C	13	9	CEC ($cmol_c$ kg^{1})	4.5	8
Total N	1.02	10	pH	6.2	2
Particulate organic C	2.94	12	——— mg kg^{-1} ———		
Particulate organic N	0.16	15	P	97	14
——— mg kg^{-1} ———			K	38	12
Inorganic N	7	7	Ca	489	8
Soil microbial biomass C	668	12	Mg	155	5
C mineralization (24 d^{-1})	521	18	S	9	15
N mineralization (24 d^{-1})	51	17	Mn	4	14
Flush of CO_2 (3 d^{-1})	197	36	Zn	7	9
			Cu	0.52	15
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	16	14	14	4
Precipitation (mm)	97	291	222	230	24

Site 15-07; Butner Beef Cattle Center-1C, Durham County, NC

- Lignum silt loam, 2 to 6 percent slopes (Fine, mixed, semiactive, thermic Aquic Hapludults); Georgeville silt loam, 2 to 6 percent slopes (Fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+ fescue, >10 years pasture
- Soil sampled 3 Sep, fertilized 3 Sep 2015, harvested 17 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	1508^{a}	29.9^{b}	12.5^{b}	74.2^{b}
45	1769^{a}	40.2^{ba}	14.2^{ba}	74.7^{b}
90	1670^{a}	40.2^{ba}	15.0^{a}	76.8^{a}
135	1855^{a}	45.4^{a}	15.5^{a}	75.8^{ba}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1458	21.9
Residue	3669	15.5

Property	**Mean**	**CV (%)**
Apparent nitrification (%)	97	1
——— g kg^{-1} ———		
Total C	24	25
Total N	2.27	6
Particulate organic C	5	22
Particulate organic N	0.28	28
——— mg kg^{-1} ———		
Inorganic N	14	14
Soil microbial biomass C	1469	15
C mineralization (24 d^{-1})	1022	10
N mineralization (24 d^{-1})	113	9
Flush of CO_2 (3 d^{-1})	343	8

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	083	59
Humic matter (g kg^{-1})	3.4	14
CEC (cmol$_{c}$ kg^{1})	13.7	6
pH	6.1	4
——— mg kg^{-1} ———		
P	74	25
K	101	16
Ca	1616	8
Mg	495	10
S	21	4
Mn	64	6
Zn	8	9
Cu	3.08	9
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	14	12	13	3
Precipitation (mm)	79	89	222	253	19

Site 15-08; Butner Beef Cattle Center-NF, Durham County, NC

- Helena sandy loam, 2 to 6 percent slopes (Fine, mixed, semiactive, thermic Aquic Hapludults)
- Ocassionally grazed, Novel E+ fescue, 1 year pasture
- Soil sampled 3 Sep, fertilized 3 Sep 2015, harvested 17 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	718.8^{a}	9.7^{c}	8.5^{c}	68.9^{c}
45	1417^{b}	21.2^{b}	9.4^{b}	73.8^{b}
90	1710^{bc}	28.4^{a}	10.4^{a}	76.6^{a}
135	2047^{c}	34.8^{a}	10.6^{a}	78.1^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1126	19.1
Residue	2850	13.0

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	84	24	Bulk density (Mg m^{-3})	1.25	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	2.4	8
Total C	14.7	1	CEC (cmol$_c$ kg^{1})	6.8	4
Total N	1.31	1	pH	6.5	1
Particulate organic C	3.82	10	——— mg kg^{-1} ———		
Particulate organic N	0.24	16	P	71	6
——— mg kg^{-1} ———			K	43	30
Inorganic N	7	21	Ca	819	3
Soil microbial biomass C	804	3	Mg	244	4
C mineralization (24 d^{-1})	512	4	S	12	3
N mineralization (24 d^{-1})	44	14	Mn	24	6
Flush of CO_2 (3 d^{-1})	135	23	Zn	3	7
			Cu	0.6	22
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	14	12	13	3
Precipitation (mm)	79	89	222	253	19

Site 15-09; LBD, Granville County, NC

- Georgeville silt loam, 2 to 6% slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed + mowed, E+; mixed fescue, 28 years pasture
- Soil sampled 18 Aug, fertilized 3 Sep 2015, harvested 16 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	1542^{a}	26.8^{b}	10.7^{c}	55.2^{b}
45	1973^{a}	36.7^{ba}	11.6^{bc}	60.0^{ba}
90	2121^{a}	42.4^{a}	12.5^{ba}	62.7^{a}
135	2046^{a}	45.0^{a}	13.7^{a}	62.7^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	3590	16.9
Residue	2506	13.8

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	58	30	Bulk density (Mg m^{-3})	1.29	3
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.70	18
Total C	20.5	8	CEC (cmol$_c$ kg^{1})	10.8	18
Total N	1.71	8	pH	6.0	6
Particulate organic C	4.30	21	——— mg kg^{-1} ———		
Particulate organic N	0.20	25	P	35	33
——— mg kg^{-1} ———			K	91	22
Inorganic N	9	35	Ca	1289	11
Soil microbial biomass C	1655	16	Mg	346	25
C mineralization (24 d^{-1})	999	4	S	18	14
N mineralization (24 d^{-1})	88	20	Mn	177	42
Flush of CO_2 (3 d^{-1})	350	10	Zn	4	7
			Cu	2.1	8
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	15	13	12	3
Precipitation (mm)	173	104	169	207	19

Site 15-10; RJ, Person County, NC

- Herndon loam, 2 to 6% slopes (fine, kaolinitic, thermic Typic Kanhapludults); Enon fine sandy loam, 6 to 10 % slopes (fine, mixed, active, thermic Ultic Hapludalfs)
- Rotationally grazed, E+ fescue, >10 years pasture
- Soil sampled 18 Aug, fertilized 3 Sep 2015, harvested 16 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	1409^{b}	28.8^{b}	12.8^{b}	63.1^{b}
45	1601^{b}	34.0^{b}	13.2^{ba}	64.7^{ba}
90	1850^{a}	39.5^{a}	13.3^{ba}	66.0^{a}
135	1905^{a}	42.5^{a}	13.9^{a}	66.6^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2026	15.7
Residue	3375	15.3

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	84	6	Bulk density (Mg m^{-3})	1.14	4
——— g kg^{-1} ———			Humic matter (g kg^{-1})	54	13
Total C	28.1	6	CEC (cmol$_c$ kg^{1})	9.8	4
Total N	2.48	7	pH	5.6	1
Particulate organic C	5.50	16	——— mg kg^{-1} ———		
Particulate organic N	0.26	24	P	101	3
——— mg kg^{-1} ———			K	112	24
Inorganic N	13	26	Ca	1001	7
Soil microbial biomass C	1384	17	Mg	320	6
C mineralization (24 d^{-1})	1159	11	S	25	8
N mineralization (24 d^{-1})	126	19	Mn	34	5
Flush of CO_2 (3 d^{-1})	432	14	Zn	6	6
			Cu	1.3	11
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	13	11	11	2
Precipitation (mm)	NA	NA	NA	NA	NA

Site 15-11; Upper Piedmont Research Station-Grazed, Rockingham County, NC

- Rhodhiss sandy loam, 2 to 8% slopes (fine-loamy, mixed, semiactive, mesic Typic Hapludults)
- Ocassionally grazed, E+ fescue, >50 years pasture
- Soil sampled 21 Aug, fertilized 3 Sep 2015, harvested 21 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	2184^{a}	44.4^{b}	12.7^{b}	54.5^{ba}
45	2090^{a}	43.7^{b}	13.1^{b}	52.2^{b}
90	2101^{a}	46.5^{ba}	13.8^{ba}	55.8^{ba}
135	2132^{a}	50.5^{a}	14.8^{a}	56.2^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	3394	20.4
Residue	2181	11.0

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	89	3	Bulk density (Mg m^{-3})	1.23	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	2.5	54
Total C	21.0	25	CEC (cmol$_{c}$ kg^{1})	14.0	35
Total N	2.21	9	pH	7.0	9
Particulate organic C	3.70	16	——— mg kg^{-1} ———		
Particulate organic N	0.22	20	P	114	16
——— mg kg^{-1} ———			K	162	19
Inorganic N	18	54	Ca	2508	44
Soil microbial biomass C	1016	4	Mg	97	19
C mineralization (24 d^{-1})	742	4	S	18	9
N mineralization (24 d^{-1})	89	12	Mn	36	15
Flush of CO_2 (3 d^{-1})	292	4	Zn	5	3
			Cu	1.3	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average Temperature (°C)	22	15	12	12	2
Precipitation (mm)	0	167	6	177	35

Site 15-12; Upper Peidmont Research Station-Mowed, Rockingham County, NC

- Casville sandy loam, 2 to 8% slopes (fine, mixed, semiactive, mesic Typic Hapludults)
- Mowed, E+ mixed fescue, >25 years pasture
- Soil sampled 21 Aug, fertilized 3 Sep 2015, harvested 19 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	922^{c}	20.4^{c}	13.8^{a}	78.3^{a}
45	1241^{b}	27.6^{b}	13.9^{a}	80.1^{a}
90	1459^{a}	35.1^{a}	15.0^{a}	80.0^{a}
135	1467^{a}	35.8^{a}	14.9^{a}	81.1^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1324	26.9
Residue	3050	17.5

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	68	13	Bulk density (Mg m^{-3})	1.15	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.4	7
Total C	26.16	11	CEC (cmol$_{c}$ kg^{1})	8.5	13
Total N	2.40	10	pH	5.7	1
Particulate organic C	2.99	30	——— mg kg^{-1} ———		
Particulate organic N	0.14	37	P	78	18
——— mg kg^{-1} ———			K	166	19
Inorganic N	12	18	Ca	1036	16
Soil microbial biomass C	1297	15	Mg	170	14
C mineralization (24 d^{-1})	907	10	S	20	13
N mineralization (24 d^{-1})	128	11	Mn	20	16
Flush of CO_2 (3 d^{-1})	351	9	Zn	13	25
			Cu	1.0	16
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	15	12	12	2
Precipitation (mm)	0	167	6	177	35

Site 15-13; JM-Rosebud West, Surry County, NC

- Fairview sandy clay loam, 8 to 15% slopes, moderately eroded (fine, kaolinitic, mesic Typic Kanhapludults)
- Rotationally grazed, E+ fescue, 14 years pasture
- Soil sampled 25 Aug, fertilized 3 Sep 2015, harvested 14 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	849^{a}	16.1^{a}	10.7^{a}	56.5^{c}
45	1080^{a}	22.2^{a}	11.4^{a}	56.2^{bc}
90	1253^{a}	26.3^{a}	13.0^{a}	58.7^{ba}
135	1203^{a}	25.4^{a}	13.4^{a}	59.6^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	660	29.4
Residue	2275	14.4

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	85	4	Bulk density (Mg m^{-3})	1.19	7
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.7	25
Total C	29.4	6	CEC (cmol$_c$ kg^{1})	16.22	8
Total N	2.1	5	pH	6.9	1.44
Particulate organic C	6.30	23	——— mg kg^{-1} ———		
Particulate organic N	0.31	35	P	85	32
——— mg kg^{-1} ———			K	214	16
Inorganic N	17	14	Ca	2075	11
Soil microbial biomass C	1384	4	Mg	59	77
C mineralization (24 d^{-1})	1171	6	S	23	5
N mineralization (24 d^{-1})	135	15	Mn	31	9
Flush of CO_2 (3 d^{-1})	374	11	Zn	26	15
			Cu	2.7	24
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	14	11	8	NA
Precipitation (mm)	246	189	201	124	NA

Site 15-14; JM-Rosebud bottom, Surry County, NC

- Arkaqua loam, 0 to 2% slopes, frequently flooded (fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts)
- Rotationally grazed, E+; mixed fescue, 14 years pasture
- Soil sampled 25 Aug, fertilized 3 Sep 2015, harvested 14 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	1155^{b}	19.8^{a}	12.0^{b}	48.3^{a}
45	1234^{ba}	22.7^{a}	13.2^{b}	49.9^{a}
90	1422^{a}	30.0^{a}	13.5^{a}	50.5^{a}
135	1481^{a}	31.7^{a}	13.5^{a}	51.4^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1604	2.46
Residue	2275	1.72

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	83	8	Bulk density (Mg m^{-3})	1.18	4
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.3	33
Total C	26.9	17	CEC (cmol$_c$ kg^{1})	12	18
Total N	2.01	11	pH	6.5	1.9
Particulate organic C	5.64	19	——— mg kg^{-1} ———		
Particulate organic N	0.27	19	P	80	7
——— mg kg^{-1} ———			K	226	24
Inorganic N	13	11	Ca	1444	18
Soil microbial biomass C	1408	7	Mg	388	23
C mineralization (24 d^{-1})	1112	13	S	20	34
N mineralization (24 d^{-1})	135	14	Mn	64	34
Flush of CO_2 (3 d^{-1})	432	14	Zn	17	33
			Cu	2.3	21
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	14	11	8	NA
Precipitation (mm)	246	189	201	124	NA

Site 15-15; Upper Mountain Research Station-UF, Ashe County, NC

- Watauga loam, 25 to 45% slopes (fine-loamy, paramicaceous, mesic, Typic Hapludults)
- Rotationally grazed, E+ fescue, >40 years pasture
- Soil sampled 25 Aug, fertilized 4 Sep 2015, harvested 14 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N upake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	1002^a	20.0^a	12.5^d	65.3^a
45	1070^a	23.3^a	13.6^c	68.5^a
90	968^a	22.5^a	14.7^b	68.5^a
135	993^a	24.8^a	15.6^a	70.5^a

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2594	21.6
Residue	2519	14.4

Property	Mean	CV(%)
Apparent nitrification (%)	22	44
——— g kg^{-1} ———		
Total C	24.2	6
Total N	2.1	8
Particulate organic C	4.28	24
Particulate organic N	0.24	28
——— mg kg^{-1} ———		
Inorganic N	17	16
Soil microbial biomass C	1327	12
C mineralization (24 d^{-1})	1374	16
N mineralization (24 d^{-1})	166	18
Flush of CO_2 (3 d^{-1})	450	20

Property	Mean	CV (%)
Bulk density (Mg m^{-3})	1.01	1
Humic matter (g kg^{-1})	4	21
CEC (cmol$_c$ kg^1)	838	7
pH	5.4	1
——— mg kg^{-1} ———		
P	30	15
K	117	13
Ca	789	10
Mg	226	12
S	20	12
Mn	71	17
Zn	7	10
Cu	2.9	17
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	17	10	9	8	-1.5
Precipitation (mm)	310	181	225	211	42

Site 15-16; Upper Mountain Research Station-LF, Ashe County, NC

- Toxaway loam (fine-loamy, mixed, superactive, nonacid, mesic Cumulic Humaquepts)
- Rotationally grazed, E+; mixed fescue, >40 years pasture
- Soil sampled 25 Aug, fertilized 4 Sep 2015, harvested 14 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate ($kg\ ha^{-1}$)	**Dry matter ($kg\ ha^{-1}$)**	**N uptake ($kg\ ha^{-1}$)**	**Crude protein (%)**	**Moisture at harvest (%)**
0	596^{a}	12.4^{ba}	13.0^{c}	66.4^{a}
45	487^{a}	11.7^{b}	15.1^{b}	67.7^{a}
90	609^{a}	15.3^{ba}	15.9^{ba}	69.2^{a}
135	643^{a}	16.9^{a}	16.5^{a}	71.5^{a}

Before fertilization	**Dry matter ($kg\ ha^{-1}$)**	**N concentration ($kg\ ha^{-1}$)**
Initial biomass	2858	13.9
Residue	2319	16.5

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	84	2	Bulk density ($Mg\ m^{-3}$)	0.91	2
——— $g\ kg^{-1}$ ———			Humic matter ($g\ kg^{-1}$)	31	30
Total C	56	5	CEC ($cmol_c\ kg^{1}$)	13.6	9
Total N	4.55	4	pH	5.4	2
Particulate organic C	4.54	13	——— $mg\ kg^{-1}$ ———		
Particulate organic N	0.22	17	P	164	18
——— $mg\ kg^{-1}$ ———			K	372	12
Inorganic N	23	7	Ca	1343	12
Soil microbial biomass C	1603	6	Mg	302	15
C mineralization ($24\ d^{-1}$)	1110	8	S	32	5
N mineralization ($24\ d^{-1}$)	217	4	Mn	64	18
Flush of CO_2 ($3\ d^{-1}$)	431	3	Zn	11	16
			Cu	3.6	24
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	17	10	9	8	-1.5
Precipitation (mm)	310	181	225	211	42

Site 15-17; Piedmont Research Station-UF, Rowan County, NC

- Lloyd clay loam, 2 to 8% slopes, moderately eroded (fine, kaolinitic, thermic Rhodic Kanhapludults)
- Hayed, Novel E+ fescue, 1 year pasture
- Soil sampled 19 Aug, fertilized 4 Sep 2015, harvested 12 Jan 2016

Forage yield and nutritive value ($\geq$10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	606^{c}	11.8^{c}	11.2^{c}	66.3^{b}
45	982^{b}	20.5^{b}	12.3^{bc}	68.6^{a}
90	1156^{bc}	25.4^{b}	12.8^{ba}	69.0^{a}
135	1365^{a}	32.2^{a}	13.9^{a}	70.3^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	428	24.8
Residue	1725	9.4

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	88	2	Bulk density (Mg m^{-3})	1.30	3
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5	17
Total C	16.0	6	CEC (cmol$_c$ kg^{1})	7.7	5
Total N	1.48	6	pH	5.5	3
Particulate organic C	2.31	16	——— mg kg^{-1} ———		
Particulate organic N	0.12	19	P	180	4
——— mg kg^{-1} ———			K	100	24
Inorganic N	14	24	Ca	775	11
Soil microbial biomass C	746	11	Mg	209	9
C mineralization (24 d^{-1})	521	12	S	30	37
N mineralization (24 d^{-1})	52	9	Mn	232	11
Flush of CO_2 (3 d^{-1})	163	2	Zn	7	12
			Cu	3.8	9
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	15	12	12	3
Precipitation (mm)	77	368	200	195	48

Site 15-18; Piedmont Research Station-LF, Rowan County, NC

- Dorian fine sandy loam, 2 to 6% slopes, rarely flooded (fine, mixed, semiactive, thermic Aquic Hapludults)
- Mowed/Ocassionally grazed, E+; mixed fescue, >10 years pasture
- Soil sampled 19 Aug, fertilized 4 Sep 2015, harvested 12 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	2179^a	59.6^a	17.3^a	77.0^a
45	2450^a	67.5^a	17.3^a	74.5^a
90	2291^a	65.0^a	18.0^a	77.9^a
135	2470^a	69.7^a	17.8^a	76.3^a

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1588	22.1
Residue	4887	12.7

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	67	31	Bulk density (Mg m^{-3})	0.99	3
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.1	18
Total C	36	8	CEC (cmol$_c$ kg^{1})	13.	13
Total N	3.50	10	pH	5.9	2
Particulate organic C	4.86	22	——— mg kg^{-1} ———		
Particulate organic N	0.26	31	P	162	30
——— mg kg^{-1} ———			K	571	28
Inorganic N	47	65	Ca	1548	13
Soil microbial biomass C	2046	9	Mg	323	16
C mineralization (24 d^{-1})	1525	8	S	33	3
N mineralization (24 d^{-1})	212	11	Mn	189	18
Flush of CO_2 (3 d^{-1})	600	6	Zn	16	29
			Cu	4.2	5
			Na	0	21

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	15	12	12	3
Precipitation (mm)	77	368	200	195	48

Site 15-19; Mountain Research Station-UF, Haywood County, NC

- Braddock clay loam, 8 to 15% slopes, eroded (fine, mixed, subactive, mesic Typic Hapludults)
- Rotationally grazed, E+ fescue, >30 years pasture
- Soil sampled 24 Aug, fertilized 4 Sep 2015, harvested 10 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	848^{a}	20.4^{a}	15.0^{c}	65.3^{b}
45	841^{a}	22.7^{a}	16.8^{b}	68.5^{ba}
90	972^{a}	27.4^{a}	17.7^{bca}	68.5^{ba}
135	994^{a}	30.6^{a}	19.1^{a}	70.5^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1762	26.9
Residue	2506	17.5

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	84	2	Bulk density (Mg m^{-3})	1.09	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.8	19
Total C	35.8	2	CEC (cmol$_c$ kg^{1})	12.9	6
Total N	3.43	2	pH	5.8	2
Particulate organic C	4	26	——— mg kg^{-1} ———		
Particulate organic N	0.27	27	P	98	9
——— mg kg^{-1} ———			K	188	49
Inorganic N	11	5	Ca	1515	4
Soil microbial biomass C	1818	3	Mg	362	10
C mineralization (24 d^{-1})	1055	12	S	21	6
N mineralization (24 d^{-1})	191	7	Mn	127	8
Flush of CO_2 (3 d^{-1})	435	10	Zn	5	9
			Cu	1.7	6
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	18	13	9	10	0.2
Precipitation (mm)	141	185	280	425	71

Site 15-20; Mountain Research Station-LF, Wake County, NC

- Cullowhee-Nikwasi complex, 0 to 2% slopes, frequently flooded (coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Fluvaquentic Humudepts; coarse-loamy over sandy or sandy-skeletal, mixed, superactive, nonacid, mesic Humaquepts)
- Rotationally grazed, E+; mixd fescue, >30 years pasture
- Soil sampled 24 Aug, fertilized 4 Sep 2015, harvested 10 Jan 2016

Forage yield and nutritive value (≥10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	828^{a}	19.8^{a}	14.9^{a}	66.4^{c}
45	860^{a}	22.4^{a}	16.3^{a}	67.7^{cb}
90	990^{a}	25.2^{a}	15.9^{a}	69.2^{b}
135	869^{a}	25.3^{a}	18.2^{b}	71.5^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2750	1.60
Residue	2818	1.02

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	38	42	Bulk density (Mg m^{-3})	0.99	3
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.5	25
Total C	33	5	CEC (cmol$_{c}$ kg^{1})	15.8	6
Total N	3.11	4	pH	5.7	1
Particulate organic C	3.39	12	——— mg kg^{-1} ———		
Particulate organic N	0.16	14	P	53	3
——— mg kg^{-1} ———			K	102	17
Inorganic N	12	15	Ca	1895	9
Soil microbial biomass C	1875	8	Mg	449	8
C mineralization (24 d^{-1})	1631	5	S	23	5
N mineralization (24 d^{-1})	64	19	Mn	96	6
Flush of CO_2 (3 d^{-1})	498	1	Zn	4	15
			Cu	3.7	31
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	18	13	9	10	0.2
Precipitation (mm)	141	185	280	425	71

Site 15-21; HB, Clay County, NC

- Brasstown-Junaluska complex, 8 to 15% slope (fine-loamy, mixed, subactive, mesic Typic Hapludults)
- Rotationally grazed, E+ fescue, >40 years pasture
- Soil sampled 24 Aug, fertilized 2 Sep 2015, harvested 10 Jan 2016

Forage yield and nutritive value ($\geq$10 cm height)

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})	N uptake (kg ha^{-1})	Crude protein (%)	Moisture at harvest (%)
0	860^{a}	21.9^{b}	15.9^{b}	80.7^{a}
45	1020^{a}	26.3^{ba}	16.2^{ba}	80.8^{a}
90	818^{a}	22.4^{b}	17.3^{a}	80.8^{a}
135	1123^{a}	31.1^{a}	17.4^{a}	81.9^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2748	25.0
Residue	3731	8.7

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	82	2	Bulk density (Mg m^{-3})	1.10	4
——— g kg^{-1} ———			Humic matter (g kg^{-1})	6.8	22
Total C	42.3	8	CEC (cmol$_c$ kg^{1})	18.1	10
Total N	3.62	7	pH	6.4	4
Particulate organic C	4.90	13	——— mg kg^{-1} ———		
Particulate organic N	0.26	23	P	197	8
——— mg kg^{-1} ———			K	479	8
Inorganic N	36	16	Ca	2691	14
Soil microbial biomass C	1879	13	Mg	299	14
C mineralization (24 d^{-1})	1220	12	S	29	7
N mineralization (24 d^{-1})	173	21	Mn	50	12
Flush of CO_2 (3 d^{-1})	478	9	Zn	8	11
			Cu	7.1	30
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	15	12	11	2
Precipitation (mm)	87	115	120	323	33

Site 15-22; LC, Loudoun County, VA

- 17B Middleburg silt loam, 2 to 7% slopes (fine-loamy, mixed, mesic Ultic Hapludalfs); 81B Brumbaugh cobbly silt loam, 2 to 7% slopes (fine-loamy, mixed, semiactive, mesic Oxyaquic Hapludults); 82 B Scattersville silt loam
- Rotationally grazed, E+; mixed fescue, >40 years pasture
- Soil sampled 5 Aug, fertilized -- Sep 2015, harvested -- Jan 2016

Forage yield and nutritive value ($\geq$10 cm height)

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**Crude protein (%)**	**Moisture at harvest (%)**
0	1604^{a}	23.6^{c}	9.3^{c}	55.4^{b}
45	1957^{a}	34.3^{b}	10.9^{b}	59.7^{ba}
90	1961^{a}	38.3^{ba}	12.3^{ba}	62.0^{a}
135	2106^{a}	46.2a	13.7^{a}	63.1^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	NA	NA
Residue	1637	NA

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	89	3	Bulk density (Mg m^{-3})	1.12	2
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.1	8
Total C	20.7	8	CEC (cmol$_c$ kg^{1})	12	5
Total N	1.9	7	pH	6.6	1
Particulate organic C	3.22	16	——— mg kg^{-1} ———		
Particulate organic N	0.15	15	P	63	9
——— mg kg^{-1} ———			K	80	18
Inorganic N	8	3	Ca	1571	8
Soil microbial biomass C	1221	12	Mg	400	5
C mineralization (24 d^{-1})	1059	14	S	16	10
N mineralization (24 d^{-1})	89	10	Mn	93	59
Flush of CO_2 (3 d^{-1})	367	20	Zn	3	19
			Cu	1.8	18
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	13	11	9	1
Precipitation (mm)	17	0	29	42	3

Site 16-1; K-farm (with clover), Monongalia County, WV

- Ernest silt loam, 8 to 15% slopes (fine-loamy, mixed, superactive, mesic Aquic Fragiudults)
- Hayed, E+; mixed fescue, -- years pasture
- Soil sampled 31 Aug, fertilized 31 Aug 2016, harvested 2 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	690^{b}	1001^{a}	11.3^{c}	15.8^{a}	10.2^{c}	9.8^{c}	57.8^{c}	69.5^{b}
45	792^{ba}	931^{a}	16.3^{b}	17.5^{a}	12.8^{b}	11.7^{b}	62.6^{b}	73.4^{a}
90	844^{ba}	845^{a}	19.4^{ba}	17.3^{a}	14.3^{ba}	12.8^{ba}	67.0^{a}	72.7^{a}
135	903^{a}	842^{a}	21.4^{a}	17.8^{a}	14.8^{a}	13.3^{a}	67.7^{a}	72.8^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	988	25.4
Residue	2125	11.5

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	46	30
——— g kg^{-1} ———		
Total C	35.1	3
Total N	2.87	4
Particulate organic C	5.49	12
Particulate organic N	0.21	16
——— mg kg^{-1} ———		
Inorganic N	12	13
Soil microbial biomass C	1493	10
C mineralization (24 d^{-1})	1563	3
N mineralization (24 d^{-1})	149	10
Flush of CO_2 (3 d^{-1})	576	4

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.01	10
Humic matter (g kg^{-1})	6.2	11
CEC (cmol$_c$ kg^{1})	12.8	9
pH	6	0
——— mg kg^{-1} ———		
P	76	30
K	68	12
Ca	2073	10
Mg	69	27
S	21	8
Mn	88	11
Zn	4	22
Cu	2.6	7
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	16	9	3	NA
Precipitation (mm)	81	121	45	94	NA

Site 16-2; K-farm (no clover), Monongalia County, WV

- Ernest silt loam, 15 to 25% slopes (fine-loamy, mixed, superactive, mesic Aquic Fragiudults)
- Hayed, E+; mixed fescue, -- years pasture
- Soil sampled 31 Aug, fertilized 31 Aug 2016, harvested 2 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	790^a	1083^a	8.7^a	12.7^a	4.8^b	5.4^c	30.8^a	46.2^a
45	762^a	1164^a	8.2^a	12.8^a	5.8^b	6.6^b	34.4^a	47.4^a
90	877^a	1072^a	8.9^a	12.2^a	7.4^a	8.1^a	34.7^a	49.8^a
135	884^a	1157^a	8.7^a	13.5^a	7.9^a	8.7^a	35.1^a	48.7^a

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2186	14.8
Residue	1650	11.8

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	24	113	Bulk density (Mg m^{-3})	0.95	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	7.4	38
Total C	43.6	8	CEC (cmol$_c$ kg^{1})	14.2	7
Total N	3.29	6	pH	6.1	4
Particulate organic C	6.03	7	——— mg kg^{-1} ———		
Particulate organic N	0.21	16	P	38	15
——— mg kg^{-1} ———			K	72	10
Inorganic N	10	25	Ca	2309	10
Soil microbial biomass C	1976	10	Mg	88	7
C mineralization (24 d^{-1})	1645	9	S	21	15
N mineralization (24 d^{-1})	162	13	Mn	100	13
Flush of CO_2 (3 d^{-1})	565	12	Zn	8	7
			Cu	6	15
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	13	11	9	1
Precipitation (mm)	17	0	29	42	3

Site 16-3; SB, Pulaski County, VA

- Lowell silt loam, 7 to 15% slopes (fine, mixed, active, mesic Typic Hapludalfs)
- Rotationally grazed + Hayed, E+; mixed fescue, -- years pasture
- Soil sampled 11 Aug, fertilized 1 Sep 2016, harvested 3 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	877^{a}	1205^{a}	15.8^{c}	19.9^{a}	11.2^{d}	10.3^{b}	55.0^{b}	53.6^{b}
45	1107^{b}	1338^{a}	21.3^{b}	22.9^{ba}	12.1^{c}	10.7^{b}	56.3^{ba}	53.6^{b}
90	1071^{b}	1361^{a}	24.1^{b}	27.0^{bc}	14.1^{b}	12.4^{a}	58.1^{ba}	54.1^{b}
135	1344^{c}	1358^{a}	32.5^{a}	27.9^{c}	14.5^{a}	13.4^{a}	60.9^{a}	55.5^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1356	2.95
Residue	2569	1.13

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	51	49	Bulk density (Mg m^{-3})	1.13	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.8	8
Total C	26.6	10	CEC (cmol$_c$ kg^{1})	8	15
Total N	2.20	8	pH	5.5	4
Particulate organic C	3.61	30	——— mg kg^{-1} ———		
Particulate organic N	0.17	43	P	35	21
——— mg kg^{-1} ———			K	172	5
Inorganic N	29	43	Ca	814	24
Soil microbial biomass C	1342	12	Mg	207	28
C mineralization (24 d^{-1})	1342	14	S	21	5
N mineralization (24 d^{-1})	153	14	Mn	60	13
Flush of CO_2 (3 d^{-1})	444	7	Zn	8	13
			Cu	1.8	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	15	10	5	NA
Precipitation (mm)	44	25	33	37	NA

Site 16-4; MS (Route100), Carroll County, VA

- Myersville loam, sloping, eroded (fine-loamy, mixed, active, mesic Ultic Hapludalfs)
- Rotationally grazed, Novel E+fescue, -- years pasture
- Soil sampled 1 Sep, fertilized 1 Sep 2016, harvested 3 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	562^{a}	772^{b}	12.1^{c}	15.8^{b}	13.3^{b}	12.7^{b}	61.7^{a}	62.0^{b}
45	669^{a}	865^{ba}	14.8^{c}	18.1^{ba}	13.8^{b}	13.1^{ba}	61.6^{a}	62.2^{b}
90	928^{a}	997^{ba}	22.5^{ba}	22.3^{a}	15.3^{a}	13.8^{ba}	64.6^{a}	64.7^{a}
135	930^{a}	961^{a}	24.7^{a}	23.45^{a}	16.5^{a}	15.1^{a}	64.1^{a}	65.2^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	257	27.5
Residue	2969	10.8

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	90	4	Bulk density (Mg m^{-3})	1.33	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5	21
Total C	28.2	7	CEC (cmol$_c$ kg^{1})	10.04	15
Total N	2.31	9	pH	6	2
Particulate organic C	3.84	35	——— mg kg^{-1} ———		
Particulate organic N	0.21	43	P	51	27
——— mg kg^{-1} ———			K	103	30
Inorganic N	30	29	Ca	1111	18
Soil microbial biomass C	1321	14	Mg	343	20
C mineralization (24 d^{-1})	1005	10	S	21	7
N mineralization (24 d^{-1})	161	12	Mn	76	14
Flush of CO_2 (3 d^{-1})	408	14	Zn	19	16
			Cu	3	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	20	14	9	3	NA
Precipitation (mm)	NA	NA	NA	NA	NA

Site 16-5; MS (homeplace), Carroll County, VA

- Manor loam, steep (coarse-loamy, micaceous, mesic Typic Dystrudepts)
- Rotationally grazed, Novel E+ fescue, -- years pasture
- Soil sampled 1 Sep, fertilized 1 Sep 2016, harvested 3 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1071^{a}	1399^{a}	22.7^{a}	27.8^{b}	13.2^{c}	125^{b}	60.4^{b}	53.4^{b}
45	1061^{a}	1378^{a}	24.6^{a}	29.8^{ba}	14.4^{b}	13.6^{ab}	60.8^{b}	54.5^{b}
90	1206^{a}	1448^{a}	31.2^{a}	32.5^{ba}	16.2^{a}	14.0^{a}	63.8^{a}	58.4^{a}
135	1082^{a}	1431^{a}	28.7^{a}	34.0^{a}	16.5^{a}	14.8^{a}	65.1^{a}	59.2^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2650	31.2
Residue	3556	15.4

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	88	5	Bulk density (Mg m^{-3})	1.08	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	2.7	72
Total C	30.4	5	CEC (cmol$_{c}$ kg^{1})	13	8
Total N	2.71	3	pH	6.3	3
Particulate organic C	4.57	28	——— mg kg^{-1} ———		
Particulate organic N	0.26	29	P	55	14
——— mg kg^{-1} ———			K	209	24
Inorganic N	32	8	Ca	1463	9
Soil microbial biomass C	1556	11	Mg	449	8
C mineralization (24 d^{-1})	1296	10	S	21	7
N mineralization (24 d^{-1})	208	21	Mn	51	12
Flush of CO_2 (3 d^{-1})	527	13	Zn	15	19
			Cu	2.3	14
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	20	14	9	3	NA
Precipitation (mm)	NA	NA	NA	NA	NA

Site 16-6; MS (woodlawan), Carroll County, VA

- Mayersville loam, steep (fine-loamy, mixed, active, mesic Ultic Hapludalfs)
- Rotationally grazed, Novel E+ fescue, -- years pasture
- Soil sampled 1 Sep, fertilized 1 Sep 2016, harvested 10 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	301^{b}	822^{a}	7.5^{b}	20.8^{a}	14.6^{c}	14.0^{c}	51.2^{b}	50.8^{a}
45	402^{ba}	839^{a}	10.5^{ba}	20.3^{a}	16.7^{bc}	16.1^{cb}	53.2^{ba}	51.8^{a}
90	519^{a}	830^{a}	13.4^{a}	20.1^{a}	15.8^{ba}	15.0^{b}	52.9^{ba}	51.2^{a}
135	498^{a}	840^{a}	12.8^{a}	20.6^{a}	17.4^{a}	16.3^{a}	54.7^{a}	53.1^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1808	27.0
Residue	2544	14.1

Property	Mean	CV(%)
Apparent nitrification (%)	85	8
——— g kg^{-1} ———		
Total C	33.7	9
Total N	2.88	9
Particulate organic C	3.96	28
Particulate organic N	0.21	38
——— mg kg^{-1} ———		
Inorganic N	31	15
Soil microbial biomass C	1548	13
C mineralization (24 d^{-1})	1105	18
N mineralization (24 d^{-1})	171	17
Flush of CO_2 (3 d^{-1})	458	20

Property	Mean	CV (%)
Bulk density (Mg m^{-3})	1.15	10
Humic matter (g kg^{-1})	5.6	26
CEC ($cmol_c$ kg^{1})	10.6	2
pH	5.9	3
——— mg kg^{-1} ———		
P	63	19
K	223	18
Ca	1108	2
Mg	352	5
S	22	10
Mn	58	14
Zn	21	13
Cu	2.5	10
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	20	14	9	3	NA
Precipitation (mm)	NA	NA	NA	NA	NA

Site 16-7; Rockingham farm , Fauquier County, VA

- Mayersville silt loam, 7 to 15% slopes (fine-loamy, mixed, active, mesic Ultic Hapludalfs)
- Grazed, E+ fescue, -- years pasture
- Soil sampled 10 Aug, fertilized 31 Aug 2016, harvested 13 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1170^{b}	1905^{b}	19.4^{c}	31.4^{b}	10.5^{c}	10.2^{b}	57.3^{b}	54.2^{c}
45	1380^{ba}	2020^{ba}	25.6^{bc}	34.3^{ba}	11.7^{cb}	10.6^{ba}	60.1^{ba}	56.5^{cb}
90	1746^{a}	2348^{a}	34.5^{ba}	44.7^{a}	12.3^{b}	11.8^{ba}	61.4^{ba}	57.8^{b}
135	1672^{ba}	2103^{ba}	40.6^{a}	42.7^{a}	15.1^{a}	12.7^{a}	66.2^{a}	61.3^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2366	19.4
Residue	1919	12.4

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	95	3	Bulk density (Mg m^{-3})	1.07	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.7	51
Total C	31.4	9	CEC (cmol$_c$ kg^{1})	19.9	13
Total N	2.99	10	pH	6.4	1
Particulate organic C	4.82	33	——— mg kg^{-1} ———		
Particulate organic N	0.30	40	P	40	18
——— mg kg^{-1} ———			K	220	37
Inorganic N	13	36	Ca	3154	13
Soil microbial biomass C	1595	9	Mg	256	29
C mineralization (24 d^{-1})	1221	12	S	17	12
N mineralization (24 d^{-1})	138	38	Mn	62	10
Flush of CO_2 (3 d^{-1})	497	18	Zn	3	29
			Cu	8.4	19
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	15	8	4	NA
Precipitation (mm)	4	3	0	0	NA

Site 16-8; Willis farm, Madison County, VA

- Louisburg sandy loam, 5 to 15% slopes (coarse-loamy, mixed, semiactive, thermic Ruptic-Ultic Dystrudepts)
- Grazed, E+; mixed fescue, -- years pasture
- Soil sampled 10 Aug, fertilized 31 Aug 2016, harvested 14 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	888^{a}	1838^{a}	15.0^{a}	31.0^{a}	10.6^{b}	10.5^{a}	71.5^{ba}	64.7^{a}
45	867^{a}	1818^{a}	16.3^{a}	34.6^{a}	11.9^{ba}	11.8^{a}	72.9^{a}	67.0^{a}
90	1021^{a}	1966^{a}	17.9^{a}	37.2^{a}	10.8^{ba}	11.8^{a}	69.1^{b}	64.1^{a}
135	983^{a}	1782^{a}	20.1^{a}	33.5^{a}	12.7^{a}	12.8^{a}	70.7^{ba}	66.7^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2222	17.0
Residue	1712	13.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	90	7	Bulk density (Mg m^{-3})	1.16	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.4	7
Total C	24.6	9	CEC (cmol$_{c}$ kg^{1})	11.8	12
Total N	2.22	8	pH	5.9	1
Particulate organic C	3.99	34	——— mg kg^{-1} ———		
Particulate organic N	0.22	42	P	21	30
——— mg kg^{-1} ———			K	78	27
Inorganic N	7	5	Ca	1493	13
Soil microbial biomass C	1242	19	Mg	228	14
C mineralization (24 d^{-1})	1108	14	S	15	14
N mineralization (24 d^{-1})	75	33	Mn	12	16
Flush of CO_2 (3 d^{-1})	432	15	Zn	2	36
			Cu	1.0	32
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	16	9	4	NA
Precipitation (mm)	77	14	14	42	NA

Site 16-9; SVAREC (Improved), Augusta County, VA

- Frederick-Christian silt loams, 7 to 15% slopes, eroded (fine, mixed, semiactive, mesic Typic Paleudults; fine, mixed, semiactive, mesic Typic Hapludults)
- Grazed, E+; mixed fescue, -- years pasture
- Soil sampled 30 Aug, fertilized 30 Aug 2016, harvested 11 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1225^{b}	1536^{b}	19.0^{c}	21.8^{c}	9.7^{d}	8.9^{c}	41.2^{a}	37.5^{a}
45	1254^{b}	1557^{b}	22.7^{cb}	26.7^{b}	11.4^{c}	10.7^{b}	42.7^{a}	40.9^{a}
90	1279^{b}	1632^{ba}	26.2^{b}	30.4^{a}	12.7^{b}	11.6^{a}	43.7^{a}	40.4^{a}
135	1527^{a}	1771^{a}	33.8^{a}	33.6^{a}	13.8^{a}	11.9^{a}	44.1^{a}	39.8^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2208	18.5
Residue	2281	10.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	56	14	Bulk density (Mg m^{-3})	3.79	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.8	24
Total C	21.2	11	CEC (cmol$_c$ kg^{1})	8.8	5
Total N	1.99	9	pH	6.2	1
Particulate organic C	2.83	34	——— mg kg^{-1} ———		
Particulate organic N	0.14	32	P	54	20
——— mg kg^{-1} ———			K	137	25
Inorganic N	12	13	Ca	1097	3
Soil microbial biomass C	1301	5	Mg	232	13
C mineralization (24 d^{-1})	1177	12	S	18	7
N mineralization (24 d^{-1})	147	8	Mn	192	8
Flush of CO_2 (3 d^{-1})	427		Zn	3	12
			Cu	2	7
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	14	8	3	NA
Precipitation (mm)	160	65	32	56	NA

Site 16-10; SVAREC (unimproved), Augusta County, VA

- Frederick-Christian silt loams, 7 to 15% slopes, eroded (fine, mixed, semiactive, mesic Typic Paleudults; fine, mixed, semiactive, mesic Typic Hapludults)
- Rotationally grazed, E+; mixed fescue, >40 years pasture
- Soil sampled 13 Sep, fertilized 13 Sep 2016, harvested 11 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1328^{a}	1946^{a}	22.9^{c}	28.6^{b}	10.7^{c}	9.2^{c}	49.6^{a}	43.0^{c}
45	1278^{a}	1873^{a}	25.0^{bc}	31.3^{b}	12.2^{b}	10.4^{b}	52.9^{ab}	46.1^{b}
90	1508^{a}	1949^{a}	30.6^{ba}	32.0^{a}	12.7^{b}	11.2^{ba}	52.7^{ab}	47.7^{ba}
135	1591^{a}	1943^{a}	36.8^{a}	37.5^{a}	14.5^{a}	12.1^{a}	47.7^{b}	56.6^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2086	14.2
Residue	3219	8.7

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	65	35	Bulk density (Mg m^{-3})	1.10	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	2.8	37
Total C	22		CEC (cmol$_c$ kg^{1})	6.4	7
Total N	1.88	4	pH	5.9	1
Particulate organic C	3.46	16	——— mg kg^{-1} ———		
Particulate organic N	0.18	17	P	59	8
——— mg kg^{-1} ———			K	155	7
Inorganic N	9	11	Ca	702	12
Soil microbial biomass C	1328	8	Mg	145	17
C mineralization (24 d^{-1})	1287	6	S	19	9
N mineralization (24 d^{-1})	122	14	Mn	93	21
Flush of CO$_2$ (3 d^{-1})	421	14	Zn	3	1
			Cu	1.1	3
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	21	14	8	3	NA
Precipitation (mm)	160	65	32	56	NA

Site 16-11; NR, Goochland County, VA

- Monacan complex (fine-loamy, mixed, active, thermic Fluvaquentic Eutrudepts)
- Rotationally grazed, E+; mixed fescue, >40 years pasture
- Soil sampled 10 Aug, fertilized 30 Aug 2016, harvested 14 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1852^{a}	1881^{a}	34.0^{b}	33.0^{b}	11.6^{c}	10.9^{c}	67.6^{a}	70.7^{a}
45	2016^{a}	2033^{a}	44.8^{a}	40.5^{a}	13.8^{b}	12.5^{b}	69.3^{a}	71.4^{a}
90	1937^{a}	2002^{a}	45.7^{a}	43.2^{a}	14.7^{ba}	13.5^{a}	69.1^{a}	71.1^{a}
135	1919^{a}	2021^{a}	47.4^{a}	46.2^{a}	15.5^{a}	14.3^{a}	69.0^{a}	71.4^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2044	19.4
Residue	2869	14.8

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	89	3	Bulk density (Mg m^{-3})	1.15	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.5	11
Total C	24.4	3	CEC (cmol$_c$ kg^{1})	10.5	12
Total N	2.35	4	pH	5.9	4
Particulate organic C	4.51	50	——— mg kg^{-1} ———		
Particulate organic N	0.27	49	P	42	55
——— mg kg^{-1} ———			K	56	12
Inorganic N	8	11	Ca	1519	18
Soil microbial biomass C	1323	6	Mg	161	12
C mineralization (24 d^{-1})	1328	6	S	17	3
N mineralization (24 d^{-1})	109	34	Mn	77	13
Flush of CO$_2$ (3 d^{-1})	577	6	Zn	7	11
			Cu	3.1	22
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	16	10	5	NA
Precipitation (mm)	101	110	45	86	NA

Site 16-12; MM, Halifax County, VA

- Clifford sandy loam, 2 to 8% slopes (fine, kaolinitic, mesic Typic Kanhapludults)
- Rotationally grazed + hayed, E+; mixed fescue, 16 years pasture
- Soil sampled 10 Aug, fertilized 30 Aug 2016, harvested 15 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1387^{a}	1998^{a}	23.2^{a}	33.1^{a}	11.2^{b}	10.4^{c}	54.8^{b}	46.5^{b}
45	1352^{a}	1990^{a}	26.9^{a}	35.6^{a}	12.5^{b}	11.2^{bc}	58.6^{ba}	51.0^{a}
90	1293^{a}	1958^{a}	28.7^{a}	36.7^{a}	13.8^{a}	11.7^{ba}	60.3^{a}	51.9^{a}
135	1118^{a}	1868^{a}	25.4^{a}	37.6^{a}	14.1^{a}	12.6^{a}	57.3^{ba}	48.8^{ba}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1976	19.0
Residue	3263	10.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	72	20	Bulk density (Mg m^{-3})	1.32	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.2	32
Total C	16.81	12	CEC (cmol$_{c}$ kg^{1})	5.3	8
Total N	1.32	10	pH	5.5	2
Particulate organic C	3.57	21	——— mg kg^{-1} ———		
Particulate organic N	0.20	22	P	91	12
——— mg kg^{-1} ———			K	75	7
Inorganic N	9	11	Ca	533	15
Soil microbial biomass C	1079	9	Mg	122	16
C mineralization (24 d^{-1})	771	7	S	15	7
N mineralization (24 d^{-1})	87	21	Mn	39	3
Flush of CO$_2$ (3 d^{-1})	276	6	Zn	4	4
			Cu	1.0	10
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	16	10	5	NA
Precipitation (mm)	74	127	26	39	NA

Site 16-13; Upper Mountain Research Station, Ashe County, NC

- Toxaway loam(fine-loamy, mixed, superactive, nonacid, mesic Cumulic Humaquepts)
- Rotationally grazed, E+ fescue, n.d. years pasture
- Soil sampled 1 Sep, fertilized 1 Sep 2016, harvested 10 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	2012^{a}	2047^{a}	32.4	29.4^{b}	10.1^{b}	8.9^{c}	40.4^{a}	34.5^{a}
45	1774^{a}	2164^{a}	32.2	34.7^{ba}	11.3^{ba}	10.1^{b}	43.5^{a}	36.9^{a}
90	1820^{a}	2193^{a}	37.2	40.2^{a}	12.6^{a}	11.4^{a}	44.1^{a}	39.0^{a}
135	1689^{a}	2081^{a}	34.1	37.5^{ba}	12.5^{a}	11.3^{a}	44.1^{a}	39.7^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	3440	19.5
Residue	2544	14.1

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	33	46	Bulk density (Mg m^{-3})	0.88	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	9.8	22
Total C	53	22	CEC (cmol$_{c}$ kg^{1})	12	16
Total N	4.59	24	pH	5.2	2
Particulate organic C	5.47	13	——— mg kg^{-1} ———		
Particulate organic N	0.28	20	P	91	49
——— mg kg^{-1} ———			K	230	16
Inorganic N	29	41	Ca	1186	25
Soil microbial biomass C	2070	25	Mg	252	17
C mineralization (24 d^{-1})	1879	9	S	30	14
N mineralization (24 d^{-1})	294	36	Mn	33	29
Flush of CO_2 (3 d^{-1})	684	5	Zn	8	24
			Cu	3.1	48
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	19	13	8	3	NA
Precipitation (mm)	91	73	85	59	NA

Site 16-14; Mountain Research Station, Haywood County, NC

- Braddock clay loam, 15 to 30%, eroded, stony (fine, mixed, subactive, mesic Typic Hapludults)
- Rotationally grazed, E+ fescue, 28 years pasture
- Soil sampled 18 Aug, fertilized 2 Sep 2016, harvested 16 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	365^{a}	734^{a}	7.3^{a}	18.9^{a}	12.9^{a}	16.1^{b}	27.3^{a}	41.5^{b}
45	289^{a}	649^{a}	6.0^{a}	17.7^{a}	13.2^{a}	17.1^{ba}	29.7^{a}	46.8^{ba}
90	334^{a}	664^{a}	6.7^{a}	17.9^{a}	12.9^{a}	16.8^{a}	28.5^{a}	43.3^{ba}
135	276^{a}	657^{a}	5.8^{a}	18.4^{a}	13.0^{a}	17.5^{a}	30.1^{a}	45.3^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2236	28.2
Residue	3663	13.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	88	2	Bulk density (Mg m^{-3})	1.12	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4	26
Total C	30.3	9	CEC (cmol$_c$ kg^{1})	12	7
Total N	2.72	7	pH	6.4	2
Particulate organic C	3.06	24	——— mg kg^{-1} ———		
Particulate organic N	0.17	22	P	91	11
——— mg kg^{-1} ———			K	139	23
Inorganic N	19	42	Ca	1480	6
Soil microbial biomass C	1559	12	Mg	432	10
C mineralization (24 d^{-1})	1183	6	S	18	6
N mineralization (24 d^{-1})	162	6	Mn	145	28
Flush of CO_2 (3 d^{-1})	465	6	Zn	6	16
			Cu	2.5	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	20	14	9	5	NA
Precipitation (mm)	56	16	105	267	NA

Site 16-15; HB, Clay County, NC

- French fine sandy loam, 0 to 3% slopes, frequently flooded (fine-loamy over sandy or sandy-skeletal, mixed, active, mesic Fluvaquentic Dystrudepts)
- Rotationally grazed, E+; mixed fescue, 40 years pasture
- Soil sampled 19 Aug, fertilized 2 Sep 2016, harvested 16 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	809^{b}	1566^{a}	12.1^{b}	24.3^{b}	9.4^{c}	9.7^{c}	51.2^{b}	66.7^{a}
45	1036^{b}	1840^{a}	17.4^{b}	31.7^{ba}	10.6^{cb}	10.7^{b}	52.0^{b}	67.7^{a}
90	862^{b}	1627^{a}	16.1^{b}	31.0^{b}	11.8^{b}	11.9^{a}	51.1^{b}	68.4^{a}
135	1487^{a}	1948^{a}	31.3^{a}	38.8^{a}	13.0^{a}	12.4^{a}	59.8^{a}	71.0^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	3312	1.65
Residue	3694	1.09

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	83	12	Bulk density (Mg m^{-3})	1.16	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.5	15
Total C	31.9	7	CEC (cmol$_c$ kg^{1})	18.7	4
Total N	2.90	7	pH	6.6	0
Particulate organic C	5.14	30	——— mg kg^{-1} ———		
Particulate organic N	0.32	35	P	385	16
——— mg kg^{-1} ———			K	427	12
Inorganic N	24	47	Ca	2880	6
Soil microbial biomass C	1521	18	Mg	265	12
C mineralization (24 d^{-1})	1251	17	S	29	13
N mineralization (24 d^{-1})	180	17	Mn	113	5
Flush of CO_2 (3 d^{-1})	523	2	Zn	14	20
			Cu	9.8	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	17	11	6	NA
Precipitation (mm)	24	5	41	85	NA

Site 16-16; University of Georgia, Oconee County, WV

- Cecil soils, 0 to 2 % slopes overwash (fine, kaolinitic, thermic Typic Kahapludults)
- NA Hayed, E+; mixed fescue, -- years pasture
- Soil sampled 15 Sep, fertilized 15 Sep 2016, harvested 22 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1549^{a}	1978^{a}	27.6^{c}	35.3^{a}	11.2^{c}	11.1^{b}	47.9^{b}	44.4^{b}
45	1623^{a}	2015^{a}	32.3^{b}	37.9^{a}	12.4^{bc}	11.7^{ba}	51.4^{ba}	46.8^{ba}
90	1532^{a}	1959^{a}	32.5^{b}	39.5^{a}	13.4^{ba}	12.6^{a}	52.4^{ba}	47.3^{ba}
135	1616^{a}	1930^{a}	37.2^{a}	39.9^{a}	14.4^{a}	12.9^{a}	53.3^{a}	48.5^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2802	18.2
Residue	3981	8.5

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	76	7	Bulk density (Mg m^{-3})	0.96	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.1	44
Total C	34.6	27	CEC (cmol$_{c}$ kg^{1})	10.7	18
Total N	3	30	pH	6.1	2
Particulate organic C	6.10	10	——— mg kg^{-1} ———		
Particulate organic N	0.35	12	P	171	46
——— mg kg^{-1} ———			K	260	26
Inorganic N	12	43	Ca	1335	21
Soil microbial biomass C	1660	20	Mg	223	14
C mineralization (24 d^{-1})	1075	11	S	17	11
N mineralization (24 d^{-1})	125	18	Mn	51	19
Flush of CO_2 (3 d^{-1})	344	10	Zn	13	39
			Cu	2.9	28
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	25	20	14	9	NA
Precipitation (mm)	31	1	57	55	NA

Site 16-17; TH , Oglethorpe County, GA

- Cecil sandy loam, 6 to 10% slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+; mixed fescue, > 40 years pasture
- Soil sampled 15 Sep, fertilized 15 Sep 2016, harvested 10 Dec 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	423^a	1048^a	8.9^b	19.1^b	12.1^c	11.4^d	60.7^b	58.4^b
45	567^a	1065^a	13.3^{ba}	22.2^{ba}	14.9^b	13.0^c	66.1^a	64.1^a
90	588^a	1161^a	15.5^a	26.4^a	16.5^b	14.2^b	66.7^a	66.3^a
135	479^a	889^a	13.9^a	21.7^{ba}	18.2^a	15.3^a	67.8^a	65.0^a

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	804	18.5
Residue	3606	7.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	85	6	Bulk density (Mg m^{-3})	1.16	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.9	40
Total C	24.4	17	CEC (cmol$_c$ kg^1)	9.4	18
Total N	2	16	pH	6.2	2
Particulate organic C	5.12	26	——— mg kg^{-1} ———		
Particulate organic N	0.28	28	P	53	20
——— mg kg^{-1} ———			K	204	27
Inorganic N	11	29	Ca	1237	21
Soil microbial biomass C	1198	12	Mg	201	21
C mineralization (24 d^{-1})	863	10	S	15	13
N mineralization (24 d^{-1})	99	21	Mn	93	38
Flush of CO$_2$ (3 d^{-1})	274	11	Zn	16	16
			Cu	20	17
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	25	20	14	9	NA
Precipitation (mm)	31	1	57	55	NA

Site 16-18; WS farm, Oglethrope County, GA

- Appling coarse sandy loam, 2 to 6% slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+fescue, 3 years pasture
- Soil sampled 24 Aug, fertilized 15 Sep 2016, harvested 20 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	307^a	907^a	8.1^a	22.3^a	16.8^b	15.4^a	66.5^a	71.1^a
45	335^a	963^a	9.7^a	24.8^a	18.4^{ba}	16.1^a	65.7^a	71.7^a
90	304^a	1004^a	9.0^a	26.0^a	18.7^{ba}	16.3^a	63.3^a	69.2^a
135	331^a	844^a	10.2^a	22.4^a	19.3^a	166^a	68.0^a	70.7^a

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1038	19.6
Residue	3600	24.4

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	72	38	Bulk density (Mg m^{-3})	1.40	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.5	13
Total C	16.2	21	CEC (cmol$_c$ kg^{1})	5.6	18
Total N	0.86	15	pH	6.0	2
Particulate organic C	5.24	33	——— mg kg^{-1} ———		
Particulate organic N	0.20	33	P	85	7
——— mg kg^{-1} ———			K	139	27
Inorganic N	8	34	Ca	671	22
Soil microbial biomass C	795	30	Mg	111	22
C mineralization (24 d^{-1})	663	22	S	11	15
N mineralization (24 d^{-1})	49	28	Mn	18	13
Flush of CO_2 (3 d^{-1})	186	19	Zn	5	16
			Cu	3.5	14
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average Temperature (°C)	25	20	14	9	NA
Precipitation (mm)	31	1	57	55	NA

Site 16-19; HW, Wilkes County, GA

- Georgeville clay loam, 6 to 10% slopes, eroded (fine, kaolinitic, thermic Typic Hapludults)
- Rotationally grazed, E+ fescue, > 40years pasture
- Soil sampled 24 Aug, fertilized 15 Sep 2016, harvested 21 Dec 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	301^{a}	1121^{a}	4.5^{b}	17.4^{a}	10.0^{b}	9.8^{a}	61.6^{a}	56.4^{a}
45	343^{a}	1175^{a}	6.5^{ba}	19.9^{a}	12.2^{ba}	10.8^{a}	64.5^{a}	59.7^{a}
90	380^{a}	1149^{a}	7.2^{a}	20.0^{a}	12.7^{ba}	11.0^{a}	65.1^{a}	59.6^{a}
135	307^{a}	1066^{a}	6.3^{ba}	19.7^{a}	13.5^{a}	11.8^{a}	65.7^{a}	61.3^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	934	1.95
Residue	5219	1.49

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	69	21
——— g kg^{-1} ———		
Total C	22.1	4
Total N	2.02	7
Particulate organic C	5.34	17
Particulate organic N	0.26	22
——— mg kg^{-1} ———		
Inorganic N	20	18
Soil microbial biomass C	1453	11
C mineralization (24 d^{-1})	952	14
N mineralization (24 d^{-1})	101	27
Flush of CO_2 (3 d^{-1})	327	17

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.23	10
Humic matter (g kg^{-1})	5.6	23
CEC (cmol$_{c}$ kg^{1})	7.1	16
pH	5.8	2
——— mg kg^{-1} ———		
P	46	9
K	124	24
Ca	892	14
Mg	115	32
S	19	22
Mn	56	13
Zn	2	24
Cu	1.2	20
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	26	21	15	10	NA
Precipitation (mm)	19	37	37	70	NA

Site 16-20; Piedmont Research Station-New Field, Rowan County, NC

- Lloyd clay loam, 2 to 8% slopes, moderately eroded (fine, kaolinitic, thermic Rhodic Kanhapludults)
- Hayed, Novel E+ fescue, 2 years pasture
- Soil sampled 131 Aug, fertilized 3 Sep 2016, harvested 26 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	507^{a}	898^{a}	7.6^{c}	13.4^{b}	9.3^{c}	9.4^{c}	49.3^{c}	45.0^{c}
45	582^{a}	943^{a}	10.0^{b}	15.1^{a}	10.8^{b}	10.0^{c}	56.0^{b}	48.6^{b}
90	599^{a}	1013^{a}	11.7^{ba}	18.1^{a}	12.2^{a}	11.2^{b}	57.9^{ba}	52.4^{a}
135	607^{a}	959^{a}	13.1^{a}	18.5^{a}	13.5^{a}	12.1^{a}	59.6^{a}	53.9^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1048	19.3
Residue	2856	12.3

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	82	10	Bulk density (Mg m^{-3})	1.20	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.2	41
Total C	17.4	2	CEC (cmol$_c$ kg^{1})	7.3	16
Total N	1.53	1	pH	5.7	2
Particulate organic C	2.49	27	——— mg kg^{-1} ———		
Particulate organic N	0.14	22	P	131	5
——— mg kg^{-1} ———			K	74	20
Inorganic N	8	17	Ca	714	25
Soil microbial biomass C	991	18	Mg	209	21
C mineralization (24 d^{-1})	732	11	S	21	12
N mineralization (24 d^{-1})	73	14	Mn	229	14
Flush of CO_2 (3 d^{-1})	291	30	Zn	9	11
			Cu	3.6	12
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	7
Precipitation (mm)	76	97	16	66	135

Site 16-21; Piedmont Research Station-Old field, Rowan County, WV

- Mecklenburg clay loam, 2 to 8% slopes, moderately eroded (fine, mixed, active, termic Ultic Hapludalfs)
- Hayed, Novel E+ fescue, 5 years pasture
- Soil sampled 13 Aug, fertilized 3 Sep 2016, harvested 26 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	659^{b}	1049^{a}	8.6^{c}	12.8^{c}	8.1^{d}	7.6^{d}	51.8^{c}	48.6^{c}
45	711^{b}	1068^{a}	10.9^{c}	14.2^{cb}	9.6^{c}	8.3^{c}	56.9^{b}	51.8^{c}
90	1164^{a}	1089^{a}	20.7^{b}	17.1^{b}	11.1^{b}	9.9^{b}	58.2^{ba}	53.3^{b}
135	1285^{a}	1228^{a}	25.8^{a}	21.9^{a}	12.5^{a}	11.1^{a}	61.1^{a}	57.7^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1600	20.7
Residue	2712	10.9

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	86	3
——— g kg^{-1} ———		
Total C	21.7	9
Total N	2.08	5
Particulate organic C	3.32	19
Particulate organic N	0.21	15
——— mg kg^{-1} ———		
Inorganic N	9	16
Soil microbial biomass C	1312	8
C mineralization (24 d^{-1})	688	6
N mineralization (24 d^{-1})	87	9
Flush of CO_2 (3 d^{-1})	224	10

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.16	10
Humic matter (g kg^{-1})	1.5	49
CEC (cmol$_c$ kg^{1})	23	16
pH	7.0	1
——— mg kg^{-1} ———		
P	369	9
K	356	22
Ca	3697	15
Mg	420	26
S	14	14
Mn	163	23
Zn	30	10
Cu	7.2	8
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average Temperature (°C)	24	17	11	6	7
Precipitation (mm)	76	97	16	66	135

Site 16-22; MJ-Rosebud, Surry County, NC

- Fairfiew sandy clay loam, 8 to 15% slopes, moderately eroded (fine, kaolinitic, mesic Typic Kanhapludults)
- Rotationally grazed, E+; mixed fescue, 16 years pasture
- Soil sampled 11 Aug, fertilized 1 Sep 2016, harvested 16 Jan 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	864^{b}	1741^{a}	12.4^{c}	28.7^{a}	8.9^{b}	10.2^{b}	49.3^{c}	56.3^{c}
45	971^{ba}	1625^{a}	15.0^{bc}	28.6^{a}	9.6^{b}	10.9^{b}	51.1^{b}	58.9^{bc}
90	1142^{a}	1839^{a}	21.0^{a}	36.5^{a}	11.4^{a}	12.3^{a}	54.6^{ba}	63.3^{ba}
135	947^{ba}	1723^{a}	17.8^{ba}	34.9^{a}	11.9^{a}	12.7^{a}	60.2^{a}	64.5^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	1158	23.0
Residue	1900	13.4

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	82	11	Bulk density (Mg m^{-3})	1.19	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.8	26
Total C	26	2	CEC (cmol$_{c}$ kg^{1})	12.2	16
Total N	1.86	3	pH	6.8	2
Particulate organic C	5.83	22	——— mg kg^{-1} ———		
Particulate organic N	0.30	28	P	34	18
——— mg kg^{-1} ———			K	162	29
Inorganic N	10	9	Ca	1516	15
Soil microbial biomass C	1333	13	Mg	449	23
C mineralization (24 d^{-1})	1157	10	S	17	5
N mineralization (24 d^{-1})	142	21	Mn	27	7
Flush of CO_2 (3 d^{-1})	390	11	Zn	20	11
			Cu	1.4	21
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	12	5	6
Precipitation (mm)	38	8	22	27	64

Site 16-23; MJ-Bar optima, Surry County, NC

- Fairview sandy clay loam, 8 to 15% slopes, moderately eroded (fine, kaolinitic, mesic Typic Kanhapludults)
- Rotationally grazed, Novel E+ fescue, 2 years pasture
- Soil sampled 11 Aug, fertilized 1 Sep 2016, harvested 16 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1334^{a}	1861^{a}	22.1^{a}	33.7^{a}	10.3^{b}	11.3^{b}	46.5^{a}	47.2^{a}
45	1386^{a}	2019^{a}	24.2^{a}	39.2^{a}	10.9^{b}	12.1^{ba}	48.1^{a}	50.2^{a}
90	1718^{a}	1765^{a}	31.4^{a}	34.1^{a}	11.3^{b}	12.0^{ba}	50.2^{a}	52.9^{a}
135	1427^{a}	1767^{a}	29.3^{a}	36.6^{a}	12.8^{a}	1.8^{a}	51.5^{a}	53.0^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	3090	20.9
Residue	3000	13.7

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	89	2	Bulk density (Mg m^{-3})	1.22	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	5.1	43
Total C	25.8	17	CEC (cmol$_c$ kg^{1})	13.9	10
Total N	2.06	16	pH	6.9	2
Particulate organic C	6.35	30	——— mg kg^{-1} ———		
Particulate organic N	0.41	33	P	149	29
——— mg kg^{-1} ———			K	214	40
Inorganic N	11	17	Ca	1884	11
Soil microbial biomass C	1547	15	Mg	413	9
C mineralization (24 d^{-1})	973	14	S	19	2
N mineralization (24 d^{-1})	124	12	Mn	32	9
Flush of CO_2 (3 d^{-1})	339	14	Zn	30	12
			Cu	5	11
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	12	5	6
Precipitation (mm)	38	8	22	27	64

Site 16-24; MJ-Behind house, Surry County, NC

- Fairview sandy clay loam, 8 to 15% slopes, moderately eroded (fine, kaolinitc, mesic Typic Kanhapludults)
- Rotationally grazed, E+; mixed fescue, >50 years pasture
- Soil sampled 11 Aug, fertilized 1 Sep 2016, harvested 16 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1359^{a}	2402^{a}	22.5^{c}	44.5^{b}	10.3^{d}	11.5^{c}	52.8^{c}	58.0^{b}
45	1484^{a}	2218^{a}	27.8^{bc}	44.4^{b}	11.6^{c}	12.5^{b}	54.8^{bc}	60.2^{cb}
90	1625^{a}	2402^{a}	32.9^{ba}	49.7^{ba}	12.6^{b}	12.9^{b}	56.6^{ba}	61.8^{cb}
135	1647^{a}	2334^{a}	36.4^{a}	52.6^{a}	13.8^{a}	14.1^{a}	58.5^{a}	63.2^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2168	26.5
Residue	2531	18.8

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	88	2
——— g kg^{-1} ———		
Total C	33.3	8
Total N	2.92	10
Particulate organic C	5.69	3
Particulate organic N	0.40	25
——— mg kg^{-1} ———		
Inorganic N	22	30
Soil microbial biomass C	1463	13
C mineralization (24 d^{-1})	1010	14
N mineralization (24 d^{-1})	170	11
Flush of CO_2 (3 d^{-1})	409	11

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.10	10
Humic matter (g kg^{-1})	5.8	21
CEC (cmol$_c$ kg^{1})	13.4	7
pH	6.4	1
——— mg kg^{-1} ———		
P	90	31
K	223	24
Ca	1557	4
Mg	468	12
S	19	6
Mn	34	12
Zn	27	11
Cu	1.7	20
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	12	5	6
Precipitation (mm)	38	8	22	27	64

Site 16-25; NL, Stanly County, NC

- Tarrus channery silty clay loam,2 to 8% slopes, moderately eroded (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+; mixed fescue, >50 years pasture
- Soil sampled 16 Aug, fertilized 3 Sep 2016, harvested 13 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	2099^{a}	2425^{a}	40.1^{ba}	47.7^{a}	11.9^{c}	12.3^{b}	61.4^{a}	58.2^{a}
45	2091^{a}	2397^{a}	43.2^{ba}	48.0^{a}	12.9^{b}	12.5^{b}	63.5^{a}	58.6^{a}
90	1873^{a}	2297^{a}	37.0^{b}	45.3^{a}	12.3^{cb}	12.3^{b}	63.6^{a}	53.8^{a}
135	2044^{a}	2355^{a}	46.5^{a}	50.7^{a}	14.2^{a}	13.4^{a}	63.8^{a}	58.6^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1621	24.9
Residue	6931	20.9

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	81	3	Bulk density (Mg m^{-3})	1.07	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.5	38
Total C	33.9	30	CEC (cmol$_{c}$ kg^{1})	19.2	8
Total N	2.92	31	pH	6.6	1
Particulate organic C	5.84	23	——— mg kg^{-1} ———		
Particulate organic N	0.33	26	P	398	16
——— mg kg^{-1} ———			K	266	11
Inorganic N	21	26	Ca	2709	10
Soil microbial biomass C	1816	8	Mg	492	13
C mineralization (24 d^{-1})	1140	8	S	22	7
N mineralization (24 d^{-1})	153	12	Mn	30	9
Flush of CO_2 (3 d^{-1})	354	20	Zn	23	14
			Cu	4	25
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	7
Precipitation (mm)	154	85	12	70	120

Site 16-26; PR, Randolph County, NC

- Georgeville silty clay laom, 2 to 8% slopes, moderately erode (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+ fescue, -- years pasture
- Soil sampled 16 Aug, fertilized 3 Sep 2016, harvested 13 Jan 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1134^{ba}	1512^{b}	20.1^{c}	29.3^{b}	11.0^{c}	12.2^{b}	38.4^{b}	38.6^{b}
45	1091^{b}	1650^{ba}	21.7^{bc}	34.2^{ba}	12.4^{b}	12.9^{a}	43.7^{a}	42.7^{a}
90	1299^{ba}	1868^{a}	26.5^{bc}	39.0^{a}	2.7^{ba}	13.0^{a}	42.6^{ba}	42.6^{a}
135	1418^{a}	1809^{ba}	30.6^{a}	38.3^{a}	13.5^{a}	13.2^{a}	44.7^{a}	43.7^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	3252	2.13
Residue	2125	1.59

Property	Mean	CV(%)	Property	Mean	CV (%)
Apparent nitrification (%)	75	35	Bulk density (Mg m^{-3})	1.24	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.6	44
Total C	19.6	12	CEC (cmol$_c$ kg^{1})	8.3	19
Total N	1.64	12	pH	6.0	1
Particulate organic C	3.74	19	——— mg kg^{-1} ———		
Particulate organic N	0.19	20	P	58	12
——— mg kg^{-1} ———			K	215	21
Inorganic N	12	17	Ca	967	26
Soil microbial biomass C	1066	35	Mg	199	24
C mineralization (24 d^{-1})	961	7	S	17	9
N mineralization (24 d^{-1})	125	12	Mn	202	11
Flush of CO_2 (3 d^{-1})	349	10	Zn	6	11
			Cu	5.2	10
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	8
Precipitation (mm)	232	122	8	80	123

Site 16-27; DY, Guilford County, NC

- Vance sandy loam, 2 to 6% slopes (fine, mixed, semiactive, thermic Typic Hapludults)
- Rotationally grazed, E+; mixed fescue, 14 years pasture
- Soil sampled 8 Sep, fertilized 8 Sep 2016, harvested 13 Jan 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	864^{a}	1240^{a}	15.7^{b}	22.9^{b}	11.4^{c}	11.5^{b}	53.1^{b}	53.5^{b}
45	793^{a}	1423^{a}	15.3^{b}	27.2^{ba}	12.2^{bc}	11.9^{ba}	55.7^{ba}	55.5^{a}
90	908^{a}	1518^{a}	18.6^{a}	30.5^{a}	12.9^{bca}	12.7^{a}	56.4^{ba}	55.2^{a}
135	845^{a}	1423^{a}	18.8^{a}	29.3^{ba}	13.9^{a}	12.9^{a}	57.4^{a}	57.3^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	3242	20.1
Residue	2856	10.4

Property	Mean	CV(%)
Apparent nitrification (%)	92	3
——— g kg^{-1} ———		
Total C	15.9	8
Total N	1.38	9
Particulate organic C	3.61	32
Particulate organic N	0.24	37
——— mg kg^{-1} ———		
Inorganic N	18	25
Soil microbial biomass C	974	11
C mineralization (24 d^{-1})	627	20
N mineralization (24 d^{-1})	86	9
Flush of CO_2 (3 d^{-1})	214	22

Property	Mean	CV (%)
Bulk density (Mg m^{-3})	1.34	10
Humic matter (g kg^{-1})	2.3	63
CEC (cmol$_c$ kg^{1})	9.1	24
pH	6.5	2
——— mg kg^{-1} ———		
P	144	28
K	70	19
Ca	1269	28
Mg	244	23
S	15	14
Mn	15	13
Zn	14	21
Cu	1.2	16
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	7
Precipitation (mm)	120	13	11	37	102

Site 16-28; JB, Rockingham County, NC

- Clover sandy clay loam, 2 to 8% slopes, moderately eroded (fine, mixed, semiactive, mesic Typic Hapludults)
- Rotationally grazed, E+; mixed fescue, >50 years pasture
- Soil sampled 2 Sep, fertilized 2 Sep 2016, harvested 18 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	541^{a}	1584^{a}	8.9^{b}	28.2^{b}	10.3^{d}	11.1^{c}	75.0^{a}	68.2^{a}
45	458^{a}	1465^{a}	8.5^{b}	29.8^{ba}	11.8^{c}	12.7^{b}	75.0^{a}	67.9^{a}
90	572^{a}	1593^{a}	11.8^{a}	34.4^{a}	12.8^{b}	13.4^{ba}	76.8^{a}	70.9^{a}
135	452^{a}	1486^{a}	9.9^{ba}	33.3^{ba}	13.7^{a}	14.0^{a}	76.1^{a}	71.3^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1932	21.2
Residue	2050	16.6

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	77	18
——— g kg^{-1} ———		
Total C	31.7	9
Total N	2.98	9
Particulate organic C	6.68	48
Particulate organic N	0.46	48
——— mg kg^{-1} ———		
Inorganic N	24	76
Soil microbial biomass C	1823	8
C mineralization (24 d^{-1})	1235	9
N mineralization (24 d^{-1})	177	10
Flush of CO_2 (3 d^{-1})	431	10

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.09	10
Humic matter (g kg^{-1})	2.8	44
CEC (cmol$_c$ kg^{1})	19.8	13
pH	6.3	1
——— mg kg^{-1} ———		
P	15	11
K	372	28
Ca	2871	16
Mg	350	15
S	17	6
Mn	57	25
Zn	7	18
Cu	3	15
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	17	11	6	6
Precipitation (mm)	98	128	29	44	118

Site 16-29; Upper Piedmont Research Station, Rockingham County, NC

- Rhodhiss sandy loam, 2 to 8% slopes (fine-loamy, mixed, semiactive, mesic, Typic Hapludults)
  Rotationally grazed + mowed, E+; mixed fescue, -- years pasture
- Soil sampled 13 Aug, fertilized 2 Sep 2016, harvested 26 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1215^{a}	1891^{a}	26.0^{b}	37.9^{a}	13.3^{c}	12.5^{c}	62.8^{b}	53.8^{a}
45	1342^{a}	1902^{a}	29.8^{ba}	39.9^{a}	13.9^{bc}	13.1^{bc}	64.7^{ba}	54.8^{a}
90	1433^{a}	1984^{a}	34.4^{a}	43.3^{a}	15.1^{ba}	13.6^{ba}	66.0^{a}	56.9^{a}
135	1292^{a}	1931^{a}	33.0^{ba}	43.6^{a}	16.0^{a}	14.1^{a}	66.1^{a}	54.4^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2990	24.8
Residue	3006	18.4

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	82	20	Bulk density (Mg m^{-3})	1.16	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	2.7	45
Total C	23	7	CEC (cmol$_{c}$ kg^{1})	14	18
Total N	2.02	8	pH	7.1	5
Particulate organic C	2.52	24	——— mg kg^{-1} ———		
Particulate organic N	0.15	21	P	102	13
——— mg kg^{-1} ———			K	149	28
Inorganic N	20	42	Ca	2616	23
Soil microbial biomass C	1007	15	Mg	83	28
C mineralization (24 d^{-1})	767	5	S	17	8
N mineralization (24 d^{-1})	100	24	Mn	46	18
Flush of CO_2 (3 d^{-1})	292	2	Zn	5	4
			Cu	1.3	7
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	23	17	11	6	6
Precipitation (mm)	98	128	29	44	118

Site 16-30; RJ, Person County, NC

- Herndon laom, 2 to 6% slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+ fescue, -- years pasture
- Soil sampled 17 Aug, fertilized 30 Aug 2016, harvested 18 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1219^{b}	1719^{a}	18.2^{b}	24.8^{b}	9.3^{d}	9.0^{c}	48.1^{c}	42.7^{a}
45	1579^{ba}	1806^{a}	27.7^{a}	30.3^{ba}	10.9^{c}	10.5^{b}	53.7^{a}	45.2^{a}
90	1696^{a}	1672^{a}	33.4^{a}	29.1^{ba}	12.3^{b}	10.8^{b}	57.0^{a}	47.0^{a}
135	1618^{a}	1766^{a}	34.9^{a}	33.0^{a}	13.4^{a}	11.6^{a}	58.7^{a}	48.0^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	1908	17.5
Residue	5037	11.0

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	70	26	Bulk density (Mg m^{-3})	1.10	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	4.2	18
Total C	26.3	9	CEC (cmol$_c$ kg^{1})	10.5	29
Total N	2.18	15	pH	5.5	3
Particulate organic C	5.06	46	——— mg kg^{-1} ———		
Particulate organic N	0.23	49	P	57	20
——— mg kg^{-1} ———			K	85	34
Inorganic N	10	9	Ca	1092	35
Soil microbial biomass C	1377	25	Mg	338	43
C mineralization (24 d^{-1})	1244	13	S	19	10
N mineralization (24 d^{-1})	121	34	Mn	53	5
Flush of CO_2 (3 d^{-1})	450	15	Zn	6	31
			Cu	2	39
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	22	16	10	5	6
Precipitation (mm)	159	181	18	47	115

Site 16-31; Butner Beef Cattle Center, Durham County, WV

- Helena sandy loam, 2 to 6% slopes (fine, mixed, semiactive, mesic Aquic Hapludalfs)
- Hayed, E+ fescue, 3 years pasture
- Soil sampled 13 Aug, fertilized 30 Aug 2016, harvested 14 Jan 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	635^{b}	1616^{a}	8.4^{c}	21.8^{c}	8.3^{d}	8.4^{d}	74.2^{b}	73.5^{a}
45	789^{ba}	1580^{a}	12.7^{bc}	24.5^{bc}	10.0^{c}	9.7^{c}	75.7^{ba}	73.7^{a}
90	1087^{ba}	1738^{a}	18.9^{ba}	28.9^{ba}	10.8^{b}	10.4^{b}	76.6^{a}	74.6^{a}
135	1141^{a}	1769^{a}	22.2^{a}	32.2^{a}	12.2^{a}	11.4^{a}	77.4^{a}	75.9^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	2251	13.3
Residue	2050	10.8

Property	Mean	CV(%)
Apparent nitrification (%)	89	6
——— g kg^{-1} ———		
Total C	16.6	9
Total N	1.42	8
Particulate organic C	3.6	34
Particulate organic N	0.21	40
——— mg kg^{-1} ———		
Inorganic N	8	21
Soil microbial biomass C	820	22
C mineralization (24 d^{-1})	611	17
N mineralization (24 d^{-1})	68	18
Flush of CO_2 (3 d^{-1})	207	13

Property	Mean	CV (%)
Bulk density (Mg m^{-3})	1.16	10
Humic matter (g kg^{-1})	3.8	32
CEC (cmol$_c$ kg^{1})	6.4	10
pH	6.0	3
——— mg kg^{-1} ———		
P	70	3
K	33	1
Ca	724	13
Mg	197	16
S	13	8
Mn	20	29
Zn	3	15
Cu	0.6	15
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	7
Precipitation (mm)	170	146	26	40	85

Site 16-32; LD, Granville County, NC

- Georgeville silt loam, 2 to 6% slopes (fine, kaolinitic, thermic Typic Kanhapludults)
- Rotationally grazed, E+; mixed fescue, >50 years pasture
- Soil sampled 17 Aug, fertilized 30 Aug 2016, harvested 16 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1176^{b}	2131^{b}	19.2^{c}	35.4^{c}	10.2^{d}	10.4^{c}	51.6^{b}	48.1^{b}
45	1146^{b}	2463^{ba}	20.9^{cb}	44.9^{b}	11.4^{c}	11.4^{b}	53.6^{b}	49.9^{b}
90	1279^{b}	2263^{ba}	24.7^{b}	44.1^{b}	12.2^{b}	12.1^{a}	57.5^{a}	53.4^{a}
135	1646^{a}	2619^{a}	34.8^{a}	52.4^{a}	13.2^{a}	12.5^{a}	58.9^{a}	54.0^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	3978	17.0
Residue	3075	13.7

Property	**Mean**	**CV(%)**
Apparent nitrification (%)	84	18
——— g kg^{-1} ———		
Total C	19.9	20
Total N	1.67	19
Particulate organic C	3.96	23
Particulate organic N	0.21	23
——— mg kg^{-1} ———		
Inorganic N	15	11
Soil microbial biomass C	1010	18
C mineralization (24 d^{-1})	834	13
N mineralization (24 d^{-1})	84	45
Flush of CO_2 (3 d^{-1})	311	16

Property	**Mean**	**CV (%)**
Bulk density (Mg m^{-3})	1.39	10
Humic matter (g kg^{-1})	2.4	63
CEC (cmol$_c$ kg^{1})	7.2	24
pH	5.7	2
——— mg kg^{-1} ———		
P	49	22
K	188	19
Ca	733	31
Mg	208	32
S	16	8
Mn	85	16
Zn	5	29
Cu	1.8	24
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	17	11	6	6
Precipitation (mm)	174	156	26	51	106

Site 16-33; LB, Pender County, NC

- Lumbee fine sandy loam, occasionally flooded (fine-loamy over sandy or sandy-skeletal, siliceous, subactive, Typic Endoaquualts)
- Grazed; Novel + fescue, 1 year pasture
- Soil sampled 12 Aug, fertilized 9 Sep 2016, harvested 12 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	780^{b}	1567^{b}	17.4^{b}	32.1^{b}	13.9^{b}	12.8^{b}	68.6^{b}	59.5^{b}
45	1285^{a}	1898^{a}	27.7^{a}	38.9^{a}	13.6^{b}	12.8^{b}	73.8^{a}	65.7^{a}
90	1078^{ba}	1340^{ba}	27.7^{a}	39.4^{a}	16.4^{a}	14.3^{a}	74.3^{a}	66.2^{a}
135	937^{ba}	1696^{ba}	25.8^{a}	41.1^{a}	17.2^{a}	15.1^{a}	75.0^{a}	66.8^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	2108	20.4
Residue	2856	12.0

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	67	16	Bulk density (Mg m^{-3})	3.87	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	34.8	4
Total C	37.8	8	CEC (cmol$_{c}$ kg^{1})	11.2	9
Total N	2.11	5	pH	6	2
Particulate organic C	4.21	27	——— mg kg^{-1} ———		
Particulate organic N	0.16	31	P	88	26
——— mg kg^{-1} ———			K	98	30
Inorganic N	10	9	Ca	1538	11
Soil microbial biomass C	1130	16	Mg	178	9
C mineralization (24 d^{-1})	622	17	S	17	4
N mineralization (24 d^{-1})	91	26	Mn	7	6
Flush of CO_2 (3 d^{-1})	244	29	Zn	8	4
			Cu	2.6	13
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	19	11	8	9
Precipitation (mm)	211	236	13	112	96

Site 16-34; Cherry Research Farm (Swine), Wayne County, NC

- Lumbee sandy loam (fine-loamy over sandy or sandy skeletal, siliceous, subactive, thermic Typic Endoaquults))
- Hayed, E+ fescue, 3 years pasture
- Soil sampled 12 Aug, fertilized 3 Sep 2016, harvested 12 Jan 2016

N fertilizer rate (kg ha^{-1})	**Dry matter (kg ha^{-1})**		**N uptake (kg ha^{-1})**		**Crude protein (%)**		**Moisture at harvest (%)**	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	664^{ac}	1173^{a}	11.1^{c}	16.9^{b}	12.0^{c}	10.3^{b}	55.0^{b}	46.7^{c}
45	648^{c}	1317^{a}	12.0^{cb}	21.7^{a}	13.3^{b}	11.9^{b}	57.2^{b}	50.3^{b}
90	790^{ca}	1320^{a}	14.7^{b}	22.2^{a}	13.4^{b}	12.0^{b}	57.0^{b}	51.9^{b}
135	853^{a}	1285^{a}	18.3^{a}	24.0^{a}	15.4^{a}	13.5^{a}	61.5^{a}	56.2^{a}

Before fertilization	**Dry matter (kg ha^{-1})**	**N concentration (kg ha^{-1})**
Initial biomass	623	27.3
Residue	2062	11.7

Property	**Mean**	**CV(%)**	**Property**	**Mean**	**CV (%)**
Apparent nitrification (%)	83	17	Bulk density (Mg m^{-3})	1.33	10
——— g kg^{-1} ———			Humic matter (g kg^{-1})	3.7	26
Total C	13.8	8	CEC (cmol$_c$ kg^{1})	5.8	8
Total N	1.14	8	pH	5.8	1
Particulate organic C	2.18	47	——— mg kg^{-1} ———		
Particulate organic N	0.11	51	P	205	16
——— mg kg^{-1} ———			K	110	13
Inorganic N	8	6	Ca	655	11
Soil microbial biomass C	982	36	Mg	117	14
C mineralization (24 d^{-1})	543	11	S	14	6
N mineralization (24 d^{-1})	56	21	Mn	13	11
Flush of CO_2 (3 d^{-1})	193	12	Zn	15	13
			Cu	4.5	21
			Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	19	12	7	9
Precipitation (mm)	234	362	17	81	85

Site 16-35; Cherry Research Farm (Dairy), County, WV

- Leaf loam (fine, mixed, active, thermic Typic Albaquults)
- Grazed + Hayed, E+ fescue, 3 years pasture
- Soil sampled 12 Aug, fertilized 3 Sep 2016, harvested 12 Jan 2016

N fertilizer rate (kg ha^{-1})	Dry matter (kg ha^{-1})		N uptake (kg ha^{-1})		Crude protein (%)		Moisture at harvest (%)	
	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm	>10 cm	5-10 cm
0	1928^{a}	2550^{a}	36.0^{b}	47.9^{b}	11.6^{d}	11.7^{c}	60.9^{b}	46.0^{b}
45	1868^{a}	2886^{a}	39.9^{ba}	58.0^{a}	13.3^{c}	12.6^{c}	64.1^{ba}	48.2^{ba}
90	2017^{a}	3005^{a}	46.5^{a}	65.8^{a}	14.4^{b}	13.7^{b}	64.4^{ba}	47.9^{ba}
135	1850^{a}	2929^{a}	45.1^{ba}	65.9^{a}	15.2^{a}	14.0^{a}	66.1^{a}	49.0^{a}

Before fertilization	Dry matter (kg ha^{-1})	N concentration (kg ha^{-1})
Initial biomass	4306	20.7
Residue	1850	13.8

Property	Mean	CV(%)
Apparent nitrification (%)	90	6
——— g kg^{-1} ———		
Total C	15.8	11
Total N	1.31	13
Particulate organic C	2.93	28
Particulate organic N	0.16	30
——— mg kg^{-1} ———		
Inorganic N	7	17
Soil microbial biomass C	726	20
C mineralization (24 d^{-1})	497	10
N mineralization (24 d^{-1})	47	21
Flush of CO_2 (3 d^{-1})	185	8

Property	Mean	CV (%)
Bulk density (Mg m^{-3})	1.30	10
Humic matter (g kg^{-1})	4.6	12
CEC (cmol$_c$ kg^{1})	7.3	24
pH	6.1	6
——— mg kg^{-1} ———		
P	152	3
K	84	16
Ca	933	31
Mg	178	39
S	12	6
Mn	11	6
Zn	15	17
Cu	0.7	48
Na	0	0

Month	Sep	Oct	Nov	Dec	Jan
Average temperature (°C)	24	19	12	7	9
Precipitation (mm)	234	362	17	81	85

Appendix B.1. Weather data for 2015 and 2016 sites

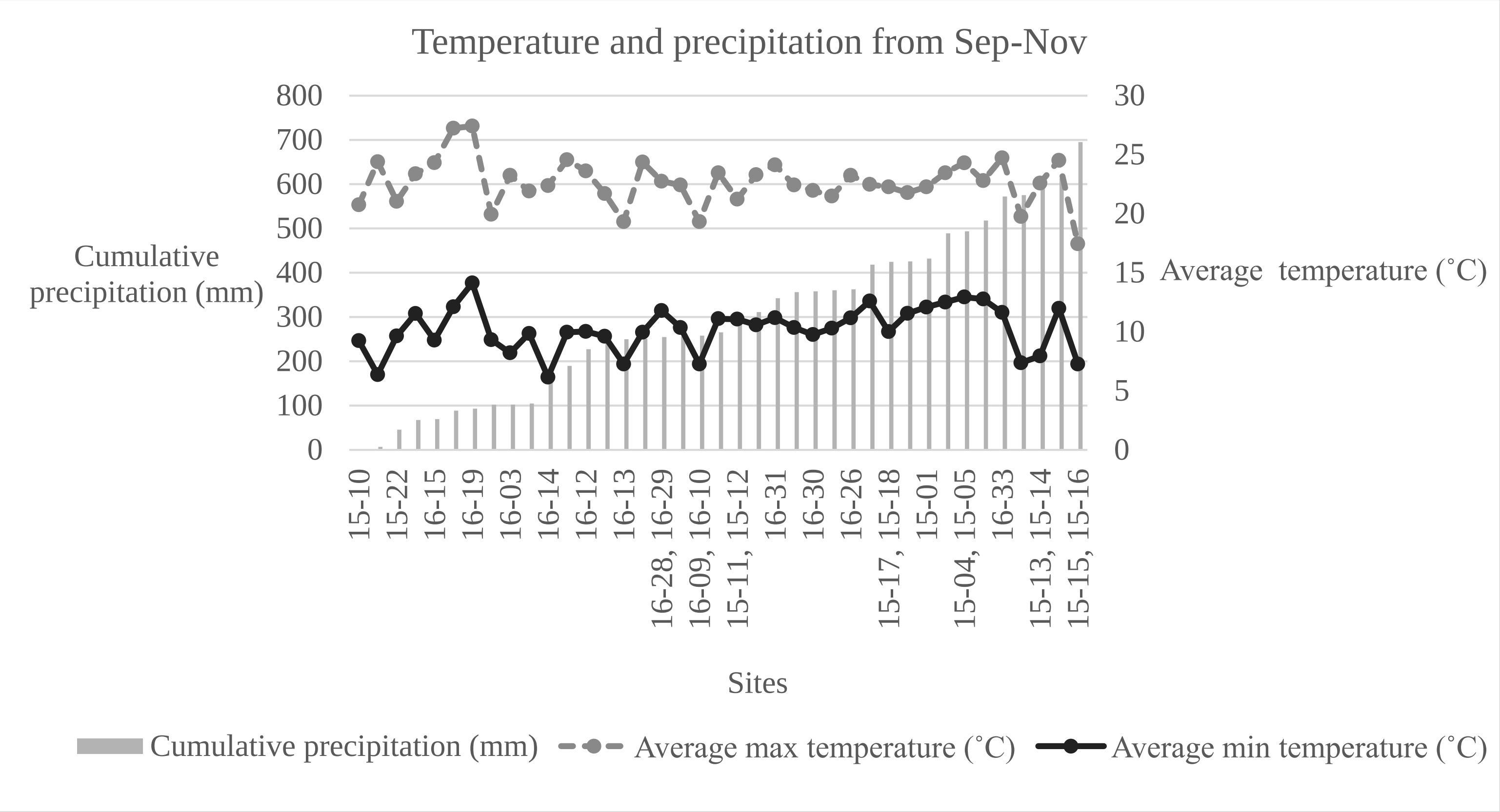


Figure B.1.Temperature and precipitation for 57 sites of study from the month of September to November.

Appendix B.2. Weather data for greenhouse trials.

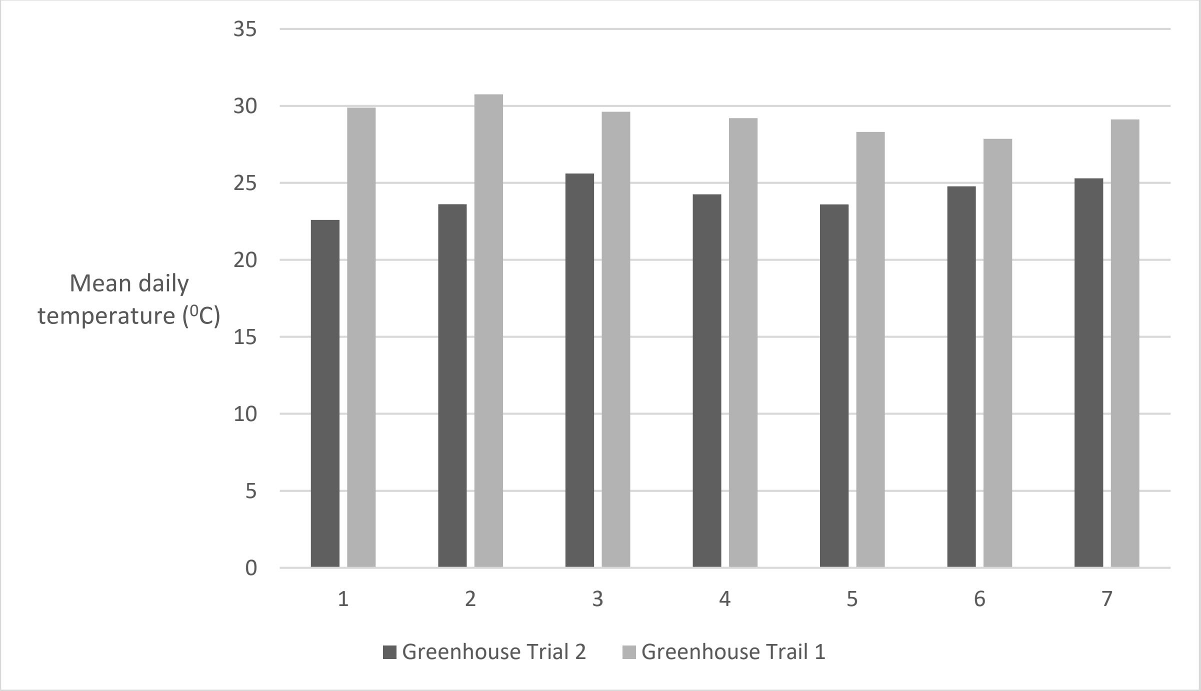


Figure B.2. Mean daily temperature for last week of greenhouse growth for Trial 1 and Trial 2.

Appendix C: Map with the sites of study

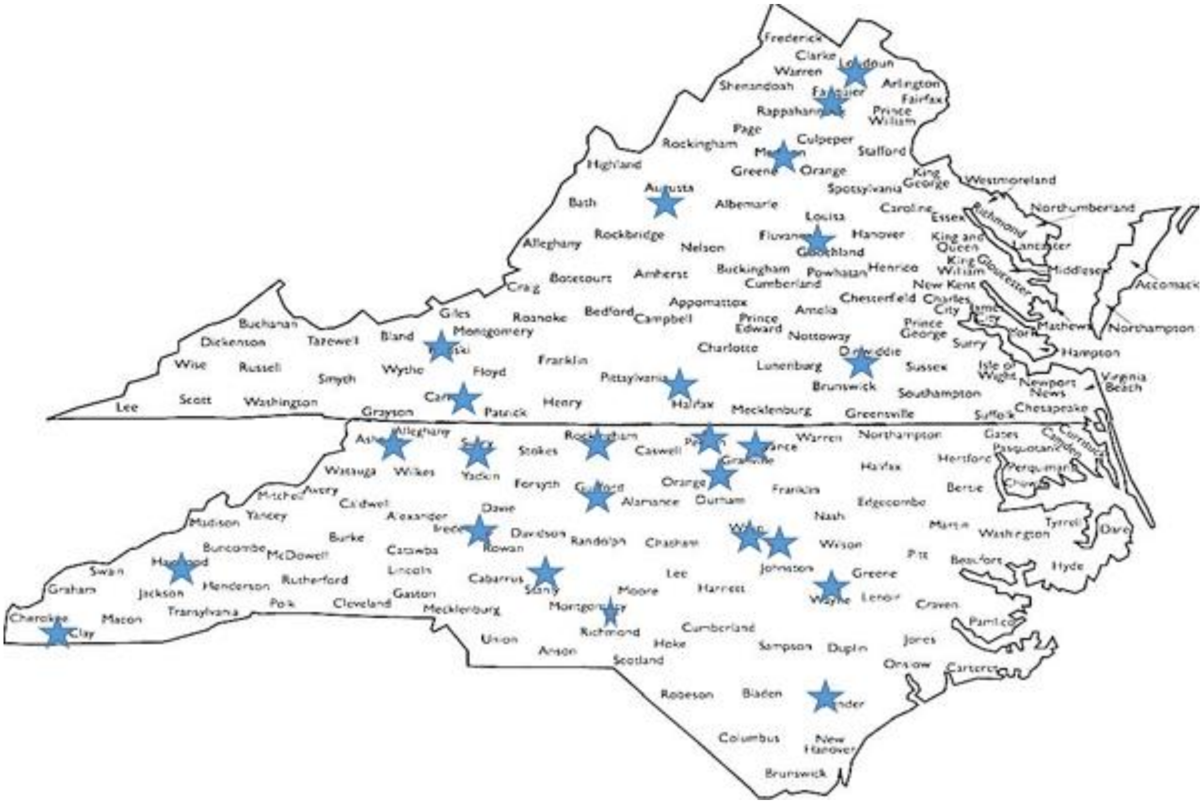


Figure C.1. Map of North Carolina and Virginia with the sites of study.

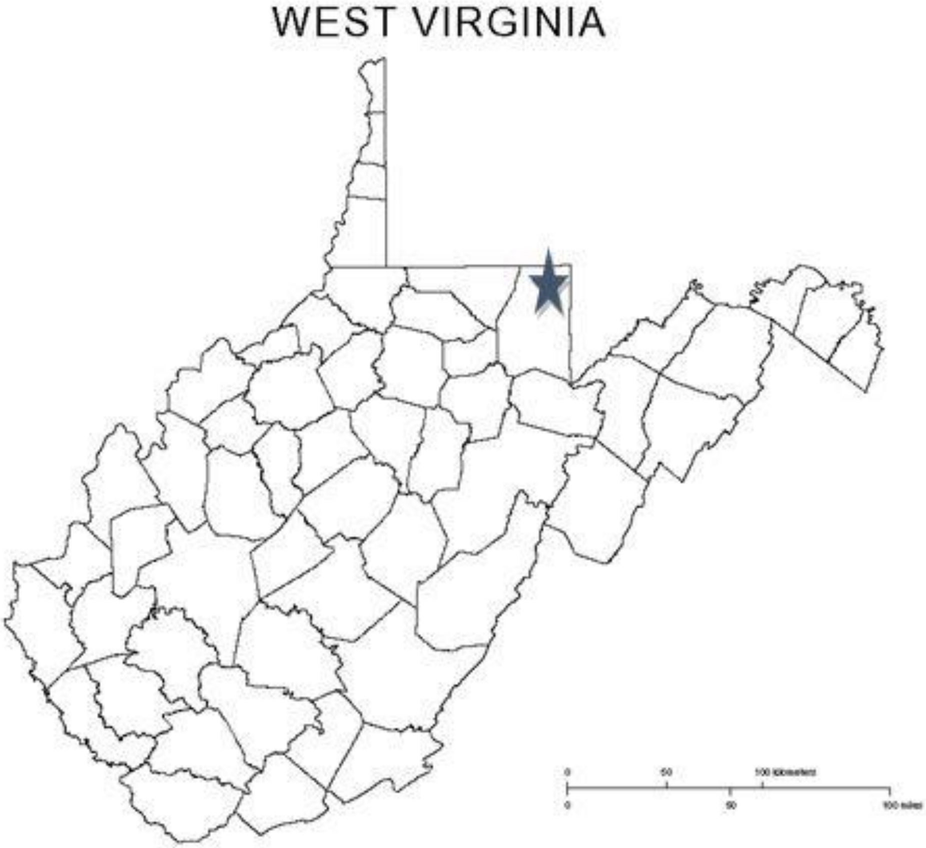


Figure C.2. Map of West Virginia with the sites of study.

Figure C.3. Map of Georgia with the sites of study.