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Effects of nitrogen and cutting management on root growth and productivity of a Kentucky bluegrass (*Poa pratensis* L.) and white clover (*Trifolium repens* L.) pasture

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**Effects of Nitrogen and Cutting Management on Root Growth and Productivity of A
Kentucky Bluegrass (*Poa pratensis* L.) and White Clover (*Trifolium repens* L.)
Pasture**

Gina Marie Kertulis

**Thesis submitted to the
College of Agriculture, Forestry and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Master of Science
In
Plant and Soil Sciences**

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Division of Plant and Soil Sciences

**Morgantown, West Virginia
2001**

**Keywords: Pasture quality, Root volume, Root dry weight, Root length, Soil nitrate
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ABSTRACT

Effects of Nitrogen and Cutting Management on Root Growth and Productivity of A Kentucky Bluegrass (*Poa pratensis* L.) and White Clover (*Trifolium repens* L.) Pasture

Gina Marie Kertulis

Nitrogen (N) and defoliation effects on productivity, root growth and soil nitrate (NO_3^-) in a Kentucky bluegrass and white clover pasture were examined. Nitrogen was applied annually in two equally split applications at rates of 0, 80 or 160 kg N ha⁻¹. After reaching 12.7 cm, plants were cut to 2.5, 5.1 or 7.6 cm. Herbage was analyzed for yield, crude protein (CP), neutral detergent fiber and acid detergent fiber. Root samples were divided into three depths: 0.0 - 5.1, 5.1 - 10.2 and 10.2 - 20.3 cm, and analyzed for volume, dry weight and length. Soil NO_3^- was measured. At the 2.5 cm cut yield and fiber concentrations were higher; CP concentration was lower. Roots in the top depth had lower volume, dry weight and length. When no N was added, CP concentration of herbage and soil NO_3^- concentration were lower, and root volume and dry weight were lower in the 0.0 – 5.1 cm depth. Cutting to 2.5 cm stopped root growth; more herbage was harvested but the material was more mature, decreasing CP and increasing fiber.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iii
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
INTRODUCTION.....	1
REVEIW OF LITERATURE.....	4
Grass and Legume Pasture Production.....	4
Effects of defoliation on pasture plants.....	4
Effects of frequency and intensity of defoliation.....	6
Forage quality and animal performance.....	7
Root Characteristics of Pasture Grasses and Legumes.....	8
Effects of defoliation on roots.....	9
Effects of nitrogen on root growth.....	10
Root distribution and water uptake.....	11
Acquisition and uptake of nutrients by roots.....	12
Root competition.....	13
Nitrogen and Pasture Production.....	14
Legumes as a source of nitrogen for pastures.....	15
Addition of nitrogen fertilizer to pastures.....	17
Effect of nitrogen fertilizer on legume populations.....	19
Legumes versus nitrogen fertilizers for pastures.....	19
Timing of nitrogen applications.....	21
MATERIALS AND METHODS.....	23
Experimental Treatments and Design.....	23
The 1999 season.....	25
The 2000 season.....	26
Pasture Production and Quality.....	27
Herbage accumulation.....	28
Total Kjeldahl nitrogen.....	28

Neutral detergent fiber and acid detergent fiber.....	28
Pasture Root Growth.....	29
Root volume.....	30
Root length.....	30
Root dry weight.....	30
Soil Nitrate.....	31
Climate Report.....	32
Statistical Analysis.....	32
RESULTS.....	34
Pasture Production and Quality.....	34
Herbage accumulation.....	34
Crude protein concentration.....	36
Crude protein yield.....	41
Neutral detergent fiber.....	42
Acid detergent fiber.....	45
Pasture Root Growth.....	47
Root volume.....	47
Root dry weight.....	51
Root length.....	54
Soil Nitrate.....	57
The 1999 season.....	57
The 2000 season.....	57
Soil Samples.....	60
Climate.....	61
DISCUSSION.....	63
Pasture Production and Quality.....	63
Effects of nitrogen fertilizer.....	63
Effects of cutting management.....	66
Pasture Root Growth.....	68
Effects of nitrogen fertilizer.....	70
Effects of cutting management.....	71
Soil Nitrate.....	72

Effects of nitrogen fertilizer.....	72
Effects of cutting management.....	73
CONCLUSIONS.....	75
LITERATURE CITED.....	78
LIST OF APPENDICES.....	88
VITA.....	101

LIST OF TABLES

Table	Page
1. Experimental treatments and treatment abbreviations.....	24
2. Treatment harvests frequencies for 1999 and 2000.....	26
3. Harvest period designations for harvest cutting dates in 1999 and 2000.....	27
4. Root sampling dates for 1999 and 2000.....	27
5. Effect of N treatment, cutting management and year on annual herbage accumulation and CP yield.....	35
6. Effect of N application, cutting management, harvest period and year on CP, NDF and ADF concentrations in herbage.....	37
7. Effect of depth, N treatment, cutting management and sampling date on root volume.....	50
8. Effect of depth, N treatment, cutting management and sampling date on root dry weight.....	52
9. Effect of depth, N treatment, cutting management and sampling date on root length.....	55
10. Effect of N treatment, cutting management and N application date on soil nitrate concentrations in 1999 and 2000.....	58
11. Effect of N treatment, cutting management and year on pH and available P, K, Ca and Mg of soil.....	61

LIST OF FIGURES

Figure	Page
1. Experimental plot layout indicating the designated sampling areas.....	24
2. Effect of year and cutting management on annual herbage accumulation.....	34
3. Effect of year and N treatment on CP concentration in herbage.....	38
4. Effect of N treatment, cutting management and year on CP concentration in herbage.....	38
5. Effect of harvest period and N treatment on CP concentration in herbage harvested in 2000.....	39
6. Effect of year and cutting management on CP concentration in herbage.....	40
7. Effect of harvest period and cutting management on the CP concentration in herbage harvested in 2000.....	40
8. Effect of year and N treatment on CP yield.....	42
9. Effect of harvest period and cutting management on NDF concentration in herbage harvested in 2000.....	44
10. Effect of year and cutting management on NDF concentration in herbage.....	44
11. Effect of year and N treatment on ADF concentration in herbage.....	46
12. Effect of year and cutting management on ADF concentration in herbage.....	46
13. Effect of harvest period and cutting management on ADF concentration in herbage harvested in 2000.....	47
14. Effect of depth and N application on root volume.....	49
15. Effect of depth and cutting management on root volume.....	49
16. Effect of depth and N treatment on root dry weight.....	53
17. Effect of depth and cutting management on root dry weight.....	54

Figure	Page
18. Effect of depth and cutting management on root length.....	56
19. Effect of N treatment, cutting management and sampling date on soil NO ₃ ⁻ concentration in 2000.....	59
20. Average monthly temperature from March to October 1999 and 2000, as recorded at Morgantown Municipal Airport.....	62
21. Total monthly rainfall from March to October 1999 and 2000, as recorded at Morgantown Municipal Airport.....	62

INTRODUCTION

The terrain that is commonly found throughout much of West Virginia is steep and mountainous. Therefore, many areas in the state are unsuitable for crop cultivation. However, permanent pastures may be a suitable and beneficial alternative agricultural land use. Pasture plants can provide soil erosion control on the steep hillsides, and the pastures, in general, can provide an economical and high quality diet for a variety of grazing animals.

In pastures in West Virginia, Kentucky bluegrass (*Poa pratensis* L.) and white clover (*Trifolium repens* L.) are considered two of the most desirable species. Both are commonly found in many of West Virginia's permanent pastures (Pierre et al., 1937). Kentucky bluegrass and white clover compliment the growth characteristics of each other and generally produce high-quality forage.

Kentucky bluegrass is an extremely palatable, cool-season grass. It is a long-lived perennial that forms a dense sod and propagates via seeds and rhizomes. It is commonly found in the northeastern and north central regions of the United States. Kentucky bluegrass is widely used because it is tolerant to high frequency and high intensity grazing and cutting; however, it is not very tolerant of high temperatures. Since Kentucky bluegrass is a cool season species it produces most of its forage by the middle of June.

White clover is a low-growing perennial legume. As opposed to Kentucky bluegrass, white clover is a short-lived species. It is valued not only for its nitrogen contributions, but for the high forage quality it produces when grown in pastures with

grasses. White clover spreads by stolons and has a rather shallow root system. Because of its shallow root system, white clover does not persist well in droughty soils. However, it is tolerant of close and frequent grazing.

In the northeastern United States, pasture production is often seriously restricted by a lack of available nitrogen (Robinson and Sprague, 1946). Nitrogen (N) deficiency in forage may reduce photosynthesis, plant growth, cell size, and protein content. Reduced crude protein is the main effect of N stress on pasture quality (Sanderson et al., 1997). The incorporation of legumes, such as white clover, into pastures is an economical method for adding nitrogen. Legumes contribute nitrogen below ground by the decay of nodules, legume root exudation, and/or a mycorrhizal fungal link between legume and grass roots (Laidlaw et al., 1996). However, white clover populations in pastures often fluctuate, and consequently its contribution of N.

Much less research has been conducted on the below ground growth of pastures. However, root growth and function are vital to the productivity and success of any plant or plant system. Roots are not simply important for their role in absorbing the necessary nutrients and water for plant survival. They may be essential for energy storage, necessary for regrowth of herbage after defoliation, and survival of the plant through the winter or drought. Root production is depends on several factors, including: genetics, environment and competition. Root competition among grass seedlings begins earlier and has a much greater impact on the overall plant production than does shoot competition. Also, root density and distribution in the soil are considered the main factors determining below ground competition (Nie et al., 1997).

This study was conducted to investigate the effects of varying N application rates and cutting management on a permanent Kentucky bluegrass and white clover pasture. The effects of the treatments on forage production and quality, root growth, and soil nitrate (NO_3^-) were examined.

The objectives of this study were:

1. To examine the effects of three N applications and three clipping managements on the crude protein and fiber content of the forage in a Kentucky bluegrass and white clover pasture.
2. To examine the effects of three N application levels and three cutting managements on root volume, root dry weight and root length, of the pasture.
3. To examine the effects of three N application rates to pasture on the levels of NO_3^- in the soil.

REVIEW OF LITERATURE

Grass and Legume Pasture Production

Proper pasture management is vital to ensure maximum production by the pasture plants as well as by the grazing animal. Several factors should be taken into consideration when managing a pasture. Some of the most important factors include: the type of plants grown in the pasture, time of year, frequency and intensity of defoliation, type of grazing animals employed, and fertilizer, if any, used on the pasture.

Each of the factors listed can influence rate of recovery after defoliation and forage quality. Pastures consisting of cool season grasses have a flush of growth in the spring season (caused by good growing conditions and the cool-season grasses entering their reproductive growth stage), a decline of growth in mid-summer, and a slight increase in production in late-summer/early-fall. Managing the frequency and intensity of defoliation is important to best utilize the abundant dry matter produced during the spring flush. For example, Williams and Hammond (1999) suggested that the pasture acreage should be divided: part used for “continuous intensive stocking” and the rest used for hay production. Management is also important during mid-summer, when growth rate is low, to avoid overgrazing and to ensure adequate availability and regrowth.

Effects of defoliation on pasture plants

Defoliation, the removal of aboveground portions of a plant, can have different effects on the entire plant and its physiology. A plant’s reaction to defoliation depends on the amount, age, rate of removal, and type of herbage removed; the plant’s environment and time of year affect a plant’s reaction to defoliation (Richards, 1993).

Generally, removal of old leaves affects a plant less than the removal of younger leaves, because older leaves are often shaded and tend to have lower photosynthetic potential (Gold and Caldwell, 1989). Also, removal of meristematic tissue, important in the production of new cells, is more detrimental to plant growth than removal of leaf tissue (Richards, 1993).

Once defoliation occurs, photosynthesis is reduced (Richards, 1993), and the plant uses its carbohydrate reserves to replace lost photosynthetic tissue (Weaver, 1930). The reduction in photosynthetic capacity affects the entire plant almost immediately. The amount of herbage removed does not necessarily correlate directly with the reduction in photosynthetic capacity of the plant. Remaining leaves (often older) have less photosynthetic capability (Richards, 1993). Remaining leaves may also experience photosynthetic rate reduction. On a defoliated alfalfa (*Medicago sativa* L.) plant undamaged leaves were found to have a 4 - 20% lower net photosynthetic rate compared to leaves on plants that were not defoliated (Hodgkinson et al., 1972; Hodgkinson, 1974).

To recover from defoliation, a plant will reallocate its resources. Photosynthate supply to roots is reduced, and the allocation of photosynthate to shoot meristems is reduced (Richards, 1993). There is an almost immediate increase in the exportation of fixed carbon from leaves that are photosynthetically active to actively growing shoots (Haratt et al., 1964; Marshall and Sagar, 1965; Ryle and Powell, 1975; Bassman and Dickmann, 1985). Also, N may be reallocated quickly within shoots. Ourry et al. (1988) found that N previously taken up by perennial ryegrass (*Lolium perenne* L.) was partitioned to regrowing leaves within 2 days after defoliation. About 80% of this N was from the remaining shoots, and the root system supplied the other 20%.

Effects of frequency and intensity of defoliation

The frequency and intensity with which a pasture is grazed affect the quantity and quality of the forage consumed by the animal, the amount of time needed for plant recovery, as well as persistence of the plants. Generally, as defoliation frequency decreases, herbage accumulation increases. Grazing height and time of year are also important. Dovel (1996) found that although lower clipping heights increased herbage mass in a wetland meadow, it had less of an effect than date of defoliation. Defoliating the stand to a height of 5 cm resulted in the highest yield, regrowth, and quality of the forage. In addition, the vigor and persistence of the stand were not visibly reduced by the lower clipping rate. In another study, Mortimer and Ahlgren (1936) found evidence to suggest that Kentucky bluegrass cut to ground level when 4 to 5 in tall, consistently yielded more than a 4 to 5 in stand defoliated to 1.5 in. Similarly, Graber (1933) found that, with abundant N fertilization, Kentucky bluegrass clipped to 0.5 in 13 times during the season yield 54% more dry matter than Kentucky bluegrass cut 13 times to 1.5 in.

Rotational and continuous grazing management may be compared in order to illustrate differences between pasture managements. Rotational grazing involves subdividing a pasture into paddocks. Animals are moved from paddock to paddock as time and/or pasture production dictates. Rotational grazing is considered to be a more intensive management and usually results in more uniform grazing. It may also reduce grazing selectivity by the animals. Continuous grazing management allows animals to graze the entire pasture continuously during the season. This allows the grazing animal to be more selective, and, depending on the number of animals, may be a less intensive management. Paine et al. (1999) examined growth and quality of rotational and

continuously grazed pastures. It was found that rotationally grazed pastures produced 1763 lb of wet herbage mass per acre per week, as compared to 850 lb herbage mass per acre per week produced by the continuously grazed pastures. The seasonal means of forage quality indicators, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were not significantly different between the two managements. However, the authors stated that rotationally grazed pastures provided forage that was higher in quality for the majority of the grazing season.

Forage quality and animal performance

Forage quality is an important factor affecting animal performance. Crude protein, NDF and ADF are often used as indicators of forage quality. Dovel (1996) examined the effects of clipping management on the yield and quality of three wetland meadows consisting of mixed forage species. Harvests were made three times during each growing season, and forage samples were analyzed for CP and ADF. Percent ADF in the bluegrass (*Poa* spp.) and clover (*Trifolium* spp.) associations were not affected by cutting height; however, percent ADF for the association did increase with harvest dates. Both harvest date and cutting height affected the crude protein content of the forage. Crude protein concentrations in the bluegrass-clover associations were inversely proportional to clipping heights. Dovel felt that the increasing CP at lower clipping heights could be explained by increased clover percentages at those heights. An increase in CP concentration was seen between the first and second harvest dates. Following a decrease in concentration between the second and third dates the CP concentration in Kentucky bluegrass was found to decline curvilinearly over the growing season (Mason and Lachance, 1983). When a bermudagrass (*Cynadon dactylon* L.) and white clover

pasture was compared to a bermudagrass pasture fertilized with $224 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, it was found that the grass and legume mixture had a higher CP concentration. Also, the amount of grass-legume pasture consumed by grazing steers was 27% greater than the amount of the N-fertilized pasture consumed, and gain per steer was significantly higher (McLaren et al., 1983).

A decrease in forage digestibility often results in reduced live weight gain (LWG) of animals. Generally, dry matter digestibility and total digestible nutrients of most forages vary inversely with lignin content (Forbes and Garrigus, 1948). Knox et al. (1958) found that clipping frequency did not affect lignin content of grass fertilized at a rate of 100 lb N ac^{-1} or less. However, at higher N fertilization rates lignin content increased as the grass aged. A study on digestibility of bermudagrass showed that average dry matter digestibility decreased in mid-summer compared to spring and fall months. In addition, daily gains of grazing steers decreased during the summer months compared to spring and autumn (Webster et al., 1965). This agrees with an earlier finding, by Elder and Murphy in 1961, that seventy percent of the weight gained by steers grazing bermudagrass pasture takes place before July 1.

Root Characteristics of Pasture Grasses and Legumes

Plant roots, although often ignored, are vital to plant survival. The roots of pasture plants are not as well studied as their aboveground counterparts. Root growth varies depending on species. Often, the main root of legumes generally endures for the life of the plant; however, lateral roots are usually “short-lived” (Lyr and Hoffman, 1967; Kolesnikov, 1971). Grasses do not have main roots but have fibrous root systems. Root

growth, as with plant growth in its entirety, is a result of both genetic makeup and environmental factors. Species, changes in hormone distribution (Lamond et al., 1983), availability of water and nutrients (Vogt et al., 1993), and soil conditions can influence structural and physiological development of root systems.

The relationships between plant roots and the soil are important. Soil conditions must allow for adequate uptake of water and nutrients to meet the needs of the plant. Also, structure and permeability of soil, soil organic matter (OM), and soil conservation are directly affected by plant root distribution and density (Gist and Smith, 1948). Plant root distribution in the soil is a result of interactions between the genetic make-up of a plant and environmental factors (Moore and Rhoades, 1966). Root distribution affects water and nutrient uptake efficiency. Root competition, grazing management, nutrient status, various environmental stresses, and plant species are only a few of the factors that influence root growth in a pasture system.

Effects of defoliation on roots

Defoliation may have several adverse effects on roots. Soon after a plant is defoliated root growth stops, and although the lifespan of roots is variable, many fine roots may die (Luo et al., 1995; Jarvis and MacDuff, 1989). There is generally a decrease in root respiration, uptake of nutrients, and N fixation in legumes (Kang and Brink, 1995). The effect on the first two, respiration and nutrient uptake, may be dependent on plant age and resource availability (Sanderson et al., 1997).

Frequency and intensity of defoliation have different effects on root growth. Root growth has been shown to stop in several C₃ forage grasses with removal of 50% or more of the shoots (Richards, 1993). A study by Kennedy and Russell, 1948, looked at the

effects of various frequencies of defoliation of a Kentucky bluegrass and white clover pasture on root biomass at different depths. Generally, the greater the frequency of defoliation, the lower the quantity of roots and rhizomes in the top three inches of soil. There was no significant difference found in root mass between 1, 4 and 8-week defoliation intervals. However, when the defoliation frequency was reduced to every 12 weeks, root production and biomass significantly increased in the top three inches.

Nitrogen fixation may be severely reduced or stopped when legumes are defoliated. In white clover, 50% defoliation has been shown to reduce the N fixation rate by 60 - 70% (Ryle et al., 1986). However, compared with many forage grasses, white clover root growth is not as affected by defoliation. Evans (1976) found that while 45% of perennial ryegrass roots died, only 5% of the white clover roots died with recurrent defoliation to a height of 2.5 cm. However, Whitehead (1982), found that with continuous and severe defoliation of white clover during flowering, plant root mass was drastically reduced, but replacement of roots that senesce due to removal of aboveground herbage, is usually rapid.

Effects of nitrogen on root growth

Nitrogen is important to plant roots because it can be used for producing and altering root biomass (Vogt et al., 1993). Nitrogen usually encourages the production of roots used for absorption (Kolesnikov, 1971). Roots normally abound in areas of higher N concentration. The form of N taken up by a plant may determine the growth pattern and lifespan of fine roots (Vogt et al., 1993). Application of N in the form of ammonium resulted in smaller root biomass and less turnover of roots compared to N in the form of

nitrate, the latter form of N supported more root biomass and more rapid root turnover (Smucker, 1984; Aber et al., 1985).

Nitrogen fertilizer may be used mainly for aboveground herbage production. This idea is supported by a study done by Holt and Fisher (1960), using Coastal Bermudagrass. Coastal Bermudagrass was treated with annual N applications ranging from 0 to 1,600 pounds per acre. Total plant mass for those plants subjected to the highest N rate was significantly higher than plants subjected to the lowest N rates. When plants were fertilized at the highest N rate, roots accounted for only 26% of the plant mass, and the root to shoot ratio was 1:2.78. For plants that received no N, roots contributed 76% of the dry weight, and the root to shoot ratio was 1:0.32. The highest N rate did not retard root growth, but it increased root growth and development only slightly.

Root distribution and water uptake

In the root zone, water content generally varies with time and space. The shallow layers in a soil profile usually are depleted of water first. Subsequent water uptake comes from increasing depths in the soil profile. The “relative distribution of roots” in a soil profile, with respect to depth, soil water retention, and water transmitting soil properties, determines the pattern of water uptake by a plant (Gardner, 1964). Overall, the distribution of a plant’s roots is thought to be much more important than the number.

Gist and Smith, in 1948, studied root development of five forage grasses in West Virginia. The experiment involved existing stands of Kentucky bluegrass, orchardgrass (*Dactylis glomerata* L.), brome grass (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and deer’s tongue (*Panicum clandestinum* L.). Core samples, 7.6 cm in diameter,

were taken from each stand and analyzed for the dry weight of roots at various depths (up to 46 cm). In the top 7.6 cm, Kentucky bluegrass had significantly more root development than did bromegrass or timothy. The high root density of Kentucky bluegrass in the top three inches of soil resulted in the buildup of a significantly higher percentage of soil OM over the course of 3 years. However, 7.6 cm, Kentucky bluegrass root biomass declined more rapidly than did the other grasses. The authors felt that because Kentucky bluegrass has a shallower root system it may be more prone to dry matter yield reductions during dry periods.

Acquisition and uptake of nutrients by roots

The acquisition of nutrients by plant roots entails several processes, including obtaining the nutrients and influencing nutrient dynamics in the rhizosphere for plant root uptake (Hinsinger, 1998). The rhizosphere is “the volume of soil influenced by root activity” (Darrah, 1993). The extent of the rhizosphere and nutrient dynamics generally varies greatly for a given nutrient and plant species. Some plant species may develop more root hairs than others (Drew and Nye, 1969) and/or develop a symbiotic mycorrhizal relationship (Bolan, 1991). Differences in soil physical properties, such as particle size, soil structure, and water content can influence the transfer of ions to the rhizosphere, as well as ion concentration gradients in the soil (Nye, 1986). Plant roots may alter soil properties. For example, roots may reduce soil porosity by radial growth (Dexter, 1987; Bruand et al., 1996), or may reduce the rhizosphere water content by uptake (Hamza and Aylmore, 1992).

Roots can induce changes in ion concentration and pH in the rhizosphere. An important role of roots is water and nutrient uptake resulting in the accumulation or

depletion of ions in the rhizosphere soil solution, true for essential, nonessential, and toxic elements. Ion concentration change is dependent on the supply of an ion as well as the plant's requirement for it (Hinsinger, 1998). Soil pH may also be affected by nutrient uptake. Generally, when nutrients are taken up by roots an imbalance of anions and cations occurs in the cells of the root. The root compensates for this charge imbalance by releasing protons when excess cations are taken up, and by releasing OH^- or HCO_3^- when excess anions are taken up. The first situation results in rhizosphere acidification, the latter in its alkalization (Haynes, 1990).

Nitrogen is an essential plant nutrient. It is also considered to be important in a plant's overall cation-anion balance, because it can be taken up as a cation, ammonium (NH_4^+), an anion, nitrate (NO_3^-), or as a molecule, N gas (N_2) (Nye, 1981). Several studies suggest that plants given ammonium acidify their rhizosphere. However, when given nitrate they alkalize their rhizosphere (Riley and Barber, 1971; Weinberger and Yee, 1984; Gahoonia et al., 1992). Legumes, which use symbiotic N_2 fixation, have been shown to compensate for excess cation uptake by acidifying their rhizosphere (Jarvis and Robson, 1983).

Root competition

Because plants in pasture systems are generally in crowded conditions, competition, not only between shoots but also between roots, can have a significant impact on root and shoot growth, and water and nutrient uptake. Competition between roots, in some instances, can affect plant growth more than shoot competition. Root density and distribution in the soil are the main factors determining below ground competition. Generally, root biomass decreases as soil depth increases (Nie et al., 1997),

but plants with greater root densities usually have a competitive advantage. Root competition may also be influenced by root hair development and characteristics, mycorrhizal associations, physiological characteristics, and/or the plant's ability to alter patterns of root growth (Caldwell, 1987). The root system of white clover does not compete as effectively as the root systems of other legumes and grasses. Root competition between white clover and other plants can be more important than shoot competition (Wilkinson and Gross, 1964).

In 1963, Donald conducted a study to examine the root competition ability between perennial ryegrass and *Phalaris tuberosa*. The two species were grown together, each having the same available soil volume. Perennial ryegrass was shown to be superior, in terms of root competition, to *Phalaris*. When the root systems of the two species were intermingled, *Phalaris* showed a greater decrease in dry matter production. However, when the shoot systems of the two species were intermingled dry matter production did not decrease as much.

Nitrogen and Pasture Production

In the northeastern United States, pasture production is often seriously restricted by a lack of available nitrogen (Robinson and Sprague, 1946). Nitrogen deficiency may reduce photosynthesis, plant growth, cell size, leaf elongation, and protein content. Under N-limiting conditions, tall fescue (*Festuca arundinacea* Schreber) was shown to have only 70% of maximum canopy gross photosynthesis (Gastal and Belanger, 1993) and increased partitioning of carbon within the plant (Belanger et al., 1992). Reduced CP concentration is the major effect of N stress on pasture quality (Sanderson et al. 1997). In

response to an N deficiency, a plant may remobilize N from other organs. In perennial grasses, this reduced supply of N may result in more fine roots at microsites with high N, as well as increased root hair density and length (Boot and Mensink, 1990; Crick and Grime, 1987).

Because grasses are unable to fix N, it is often recommended meadows and pastures be fertilized. Addition of N generally increases herbage accumulation by increasing the rate of leaf area development and light interception (Gastal and Belanger, 1993). Nitrogen fertilization may also increase forage nutritive value. The incorporation of legumes, such as white clover, into pastures, is an economical method for supplying N. Fertilization of pasture using inorganic N is another method commonly employed to increase grassland production.

Legumes as a source of nitrogen for pastures

Atmospheric N may be fixed between legumes and rhizobia and transferred to neighboring non-leguminous species. Legumes contribute N below ground by the decay of nodules, legume root exudation and/or a mycorrhizal fungal link between legume and grass roots (Laidlaw et al., 1996). The annual amount of N fixed by white clover, varying according to clover yields and mineral N availability, may range from 66 to 100 kg N ha⁻¹ yr⁻¹ in Nova Scotia, Canada, and 45 to 390 kg N ha⁻¹ yr⁻¹ in New Zealand (Caradus, 1990). Environmental factors, such as high soil nitrate levels, low soil pH, clover defoliation, high temperatures, moisture stress, low light intensity, and pests, may reduce white clover root nodulation. Also, white clover populations in pastures often fluctuate with consequent fluctuations in the N contribution from the legumes.

Dubach and Russelle (1994) cite several studies showing that only a small amount of N is released by excretion from roots and nodules, compared to the amount of N fixed from the atmosphere. Therefore, it is thought that the release of N from dead roots and nodules in grass/legume mixtures is generally the main source of transferable N (Dubach and Russelle, 1994; Laidlaw et al. 1996).

A study by Dubach and Russelle in 1994, involving established alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), attempted to quantify the origin and amount of N in nodules and fine roots. Alfalfa shoots were harvested during weeks 8 and 12 after being transplanted to a growth chamber; the shoots of birdsfoot trefoil were harvested during week 10 after being transplanted to a growth chamber. The authors found that legume root N concentration was higher in fine roots that were produced during the first 2 weeks of regrowth after harvesting, compared to fine roots produced just prior to harvest. However, the C/N ratios were lower. Also, root N content did not change over time. Dead nodules of alfalfa and birdsfoot trefoil contained about one-third of the N content of living nodules. The authors attributed this to the N being remobilized to living nodules. Total N potentially available for transfer upon root senescence ranged from 3.4 to 15.6 kg ha⁻¹ m⁻¹ yr⁻¹ for alfalfa and 1.1 to 3.5 kg ha⁻¹ m⁻¹ yr⁻¹ for birdsfoot trefoil, depending on depth. The total N potentially available for transfer upon nodule death was 0.2 to 1.8 and 0.2 to 6.6 kg ha⁻¹ yr⁻¹, depending on depth, for alfalfa and birdsfoot trefoil, respectively. The authors felt that these results support the idea that N released from decaying roots and nodules, although not the primary source of N is a major source of N for companion plants (Dubach and Russelle, 1994).

Laidlaw et al. examined the rate of N turnover from white clover roots relative to grass roots due to decomposition in 1996. A three-year-old perennial ryegrass and white clover mixed sward, continuously grazed by steers, was studied over two, 2-week periods. Subplots were treated with N^{15} , harvested, and 7.5 cm diameter by 10 cm deep root cores were extracted. Turnover was estimated as the difference between the net accumulation rate of N and the mean assimilation rate into roots for the two periods. The authors found that N turnover and assimilation in grass roots was slower and much lower than in clover roots and stolons. They estimated the loss of N from perennial ryegrass roots to be $0.7 \text{ mg N m}^{-2} \text{ d}^{-1}$ and $8.0 \text{ mg N m}^{-2} \text{ d}^{-1}$ for white clover. Since N loss estimates were made in the autumn, when it is common for root and stolon biomass to decline, the authors felt that the N turnover rate for clover would be higher for the entire grazing season. The grass root fraction, however, may have been overestimated. This is because grass roots were not the only roots included in the sample. Any roots that were not white clover roots, such as weed roots, were included with the grass root fraction.

Addition of nitrogen fertilizer to pastures

Nitrogen fertilizers are commonly added to pastures to increase herbage accumulation and forage nutritive value. Optimum rates of N vary for several reasons, including forage species, soil moisture, climate, cost of the fertilizer, and economic benefit. Numerous experiments have shown the benefits of adding N fertilizer to pastures, and have examined appropriate rate and timing of applications.

Robinson and Sprague (1946) studied the effects of N fertilizer applications and cutting frequency Kentucky bluegrass and white clover pasture. Nitrogen was applied at 40 lb ac^{-1} in early spring and after each harvest except the last fall harvest. Because

cutting frequencies varied, the total amount of N applied in a year was greater at higher cutting frequencies. Plots treated with N had higher dry matter yields than plots to which no N was applied. A similar study conducted by Swift et al. (1948) in Pennsylvania found that the lowest content of Kentucky bluegrass, in a Kentucky bluegrass/white clover pasture, was on a plot that received no fertilizer. It was found that as fertilizer applications increased, so did the Kentucky bluegrass dry matter yield. However, the yield of white clover decreased.

Raese and Decker (1966) conducted an experiment to examine how yield and stand persistence is affected by N fertilization. Orchardgrass, bromegrass, and reed canarygrass (*Phalaris arundinacea* L.) were seeded, fertilized with 25 or 75 lb N ac⁻¹, and harvested at either pre-joint, early head, early bloom, or late bloom growth stages. Each stage, except pre-joint, was cut to 1.5 or 3.5 inches. Dry matter production was determined, and stands were rated from 1 to 10 according to percent groundcover, where 10 was equal to 100% groundcover. It was found that yield increased significantly with increasing N applications. However, the higher levels of N significantly reduced the stands of orchardgrass and bromegrass, whereas reed canarygrass stands responded favorably to the high N level.

High NO₃⁻ content in forage is a potential drawback of N fertilizer application. Excess N can accumulate as NO₃⁻ when the rate of nitrate uptake exceeds the rate of protein synthesis. Slower plant growth, photosynthesis, and absorption of phosphate may result in nitrate accumulation. This accumulation may affect animal health and forage protein quality and degradability of the protein by a ruminant. Nitrate poisoning in

animals may cause rapid breathing, bluing of mucous membranes, and death due to asphyxiation (Sanderson et al., 1997).

Effect of nitrogen fertilizer on legume populations

Another potential drawback of adding N fertilizer to a mixed sward is reduced legume population. Birdsfoot trefoil and white clover, in mixed swards, have been shown to decrease when 200 to 400 lb N ac⁻¹ is added, compared to stands with no N added (Wolf and Smith, 1964). However, several factors, such as grazing frequency and height, and soil moisture, may also affect legume persistence. In a study by Robinson and Sprague (1946), white clover populations, N fertilization, defoliation, and soil moisture were examined. They found that, generally, with high N fertilization, clover populations decreased significantly. During the second year of the experiment, white clover ranged from 33 to 57% in the swards when no N was applied; with the high N application clover ranged from 1 to 60%. Clover populations were less affected by high N applications when soil moisture was adequate and when the swards were under severe clipping management. The high N and irrigated treatment that was cut to 0.5 to 1.0 in when the sward reached 3 to 5 in resulted in the highest clover population (60%).

Legumes versus nitrogen fertilizer for pastures

It is generally accepted that the addition of legumes to a pure grass stand increases dry matter yield and results in more even seasonal forage distribution (Jones, 1967). Legumes usually have higher protein percentages than do grasses. Also, grasses have increased protein content when grown in association with legumes (Churchill, 1947). This higher forage quality has been found to consistently increase output per animal (Blaser et al., 1956; Heinemann and VanKeuren, 1958; Jordon and Wedin, 1961).

Although dry matter production was the same, stands consisting of 30 to 45% legumes were found to significantly increase milk production acre^{-1} compared to stands of pure grass (Austenson et al., 1959). Similarly, a study conducted by Williams (1967) showed that the number of calves per cow and the weaning weights of the calves were greater for cows grazing grass-legume mixed pastures than for those cows grazing pastures fertilized with N.

However, it has also been shown that higher dry matter yields are produced by N fertilization of pastures. Clark et al. (1966) conducted a three-year experiment to compare N fertilized grasses and grass-legume mixtures. The treatments were compared based on forage dry matter production, pasture carrying capacity, and milk production. The four treatments were: orchardgrass-white clover mixed sward and orchardgrass with 100, 200, and 300 lb N ac^{-1} applied in four split applications annually. Two lactating cows were assigned to each replication of each treatment. Nitrogen applications significantly increased the dry matter production of the orchardgrass as compared to the orchardgrass-white clover mixture. These higher dry matter yields dictated the carrying capacity. As N rate increased, so did the CP content of the forage. Because the clover population went from 46% in year one to 3% in year three the CP and the dry matter yield of the mixed sward steadily decreased. Generally, milk production cow^{-1} was higher for the grass-clover pasture; nevertheless, as the clover population decreased, milk production cow^{-1} of the orchardgrass plus N and the mixed pasture treatments became similar. The authors concluded that white clover could be efficiently grown in association with orchardgrass for 2 to 3 years. However, they felt that, when the clover

population begins to decline, applications of up to 200 lb N ac⁻¹ could significantly increase orchardgrass dry matter yield.

Timing of nitrogen applications

Timing of N fertilizer applications plays an important role in herbage accumulation and quality of forages. Dawson and Ryden (1985) found that when 130 kg N ha⁻¹ was applied to ryegrass swards in spring, the uptake of N in mid-season was lower than swards fertilized at a rate of 40 kg N ha⁻¹. Also, at the 130 kg N ha⁻¹ rate, the ryegrass appeared to suffer greater from water stress.

Centipedegrass (*Eremochloa ophiuroides* Hack.), if subject to high levels of N at inappropriate times, may experience a decline of growth that includes slow spring greening, becoming chlorotic, and possibly death (Johnson and Carrow, 1988). Johnson et al. (1988) found that N applied at a rate of 100 kg ha⁻¹ in April and 100 kg N ha⁻¹ in September improved the quality of the centipedegrass. It also reduced the decline of the grass significantly, compared to a single application of 100 kg N ha⁻¹ in April. However, during the fourth and fifth years of the experiment, the split application of N in April and September resulted in grass of lower quality than the single application of N in April. A similar study of centipedegrass revealed that a 100 kg N ha⁻¹ split application applied in April and July resulted in higher quality grass compared to 3 to 4 split applications of N equaling 100 kg ha⁻¹ (Johnson and Carrow, 1988).

Timing may also affect pest resistance and over-wintering capability of a grass. Applying N in late autumn or close to winter may increase grass growth. This rapid growth can prevent plants from becoming winter-hardy, because plants cannot be frost hardened during rapid growth (Dexter, 1956). High N applications may affect winter

survival of grasses (Carroll and Welton, 1939). Orchardgrass fertilized with 364 to 532 kg N ha⁻¹ had a lower winter survival rate than stands of orchardgrass fertilized with 140 to 196 kg N ha⁻¹ (Howell and Jung, 1965). Sheard (1970) found that an application of 56 kg N ha⁻¹ applied to timothy in early September resulted in the greatest increase in shoot and root growth of the grass. Nitrogen applications exceeding 168 kg N ha⁻¹ generally resulted in lower herbage accumulations and growth rates.

MATERIALS AND METHODS

The experiment was carried out over a period of two years (1999 and 2000) on the West Virginia University Livestock farm near Morgantown, WV (39° 39' N latitude and 79° 55' W longitude). The area used for the experiment is a moderately sloped native pasture and has been traditionally used for the grazing of cattle. The pasture consisted mainly of Kentucky bluegrass and white clover. The plots were located on the Dormont soil series, which is a deep, moderately well drained, fine-loamy Ultic Hapludalf. The slope of the plots was 10 to 17%.

Experimental Treatments and Design

The effects of two factors were examined in the experiment (Table 1). The first factor was N fertilization. Nitrogen was applied in the form of ammonium nitrate (NH_4NO_3) at three rates: 0 kg N ha⁻¹ (0 lb N ac⁻¹), 80 kg N ha⁻¹ (71 lb N ac⁻¹), and 160 kg N ha⁻¹ (143 lb N ac⁻¹) each year, split in two equal applications. These N application times were chosen to come immediately after the peak of Kentucky bluegrass spring growth and at the beginning of its regrowth after summer. The second factor was the cutting regimen. Plots were allowed to grow to 12.7 cm (5.0 in). When they reached 12.7 cm, they were then cut to one of three heights: 2.5 cm (1.0 in), 5.1 cm (2.0 in) or 7.6 cm (3.0 in). The cutting heights represented grazing intensity. The experimental design was a randomized complete block with a 3 (nitrogen) x 3 (clipping height) factorial arrangement of treatments. Four blocks, each with 9 plots, a total of 36 plots, made up the experimental area. Each of the plots had an area of 2 x 4 m (Fig. 1). A section of

Table 1: Experimental treatments and treatment abbreviations.

Treatment	Treatment Abbreviation
N	
0 kg N ha ⁻¹	none
80 kg N ha ⁻¹	medium
160 kg N ha ⁻¹	high
Cutting	
2.5 cm (1 in)	2.5 cm
5.1 cm (2 in)	5.1 cm
7.6 cm (3 in)	7.6 cm

*There was a 53 cm (21 in) border around the perimeter of each plot.

Figure 1: Experimental plot layout indicating the designated sampling areas

60 x 80 cm within each plot and was divided into eight 15 x 40 cm sampling areas, designated for destructive sampling of roots and insertion of anion transfer membranes. Each destructive sampling area was randomly assigned to a root sampling time, one through eight. A different area was used at each sampling time. Anion transfer membranes were placed in areas assigned to the next scheduled root core sample. Herbage accumulation was measured using a plate meter (Bryan et al., 1989; Rayburn and Rayburn, 1998). Herbage accumulation was estimated by harvesting the 0.5 x 2.0 m area in each plot. The herbage and root sampling sections were randomly assigned to the top or bottom of each plot. Successive harvests were made from the same area.

The 1999 season

The area for the experiment was cut to 1.27 cm (0.5 in) using a rotary lawn mower with a bag attachment in March 1999, and the cut material was removed. Soil samples, at 0.0 - 5.1 cm (0 - 2 in) and 0.0 - 7.6 cm (0 - 3 in) depths were taken in each plot. No soil amendments were added. Plots cut to 7.6 cm were harvested three times. The 5.1 cm treatment plots were harvested twice. All of the 2.5 cm treatment plots were harvested once, and the 2.5 cm cutting treatments with medium and high nitrogen applications were cut an additional time (Table 2). The cutting dates were divided into 3 harvest periods (Table 3). Nitrogen was applied according to treatment on June 3, 1999 and September 10, 1999. Prior to both of these applications anion transfer membranes were placed in each plot to measure NO_3^- concentrations in the soil. A root core sample from each plot was taken in April, May, September and October (Table 4).

The 2000 season

In 2000, all 7.6 cm treatment plots were harvested seven times, all 5.1 cm treatment plots were harvested six times, and all 2.5 cm treatment plots were harvested four times (Table 2). The cutting dates were again divided into 3 harvest periods (Table 3). On June 2, 2000 and August 30, 2000 N was applied and anion transfer membranes were placed in each plot. Root samples were taken three times from each plot: May, August, and October (Table 4). Soil samples, at 5.1 cm (2 in) and 7.6 cm (3 in) depths were taken from each plot in October.

Table 2: Treatment harvest frequencies for 1999 and 2000.

Treatment		Harvest Frequency	
Cut	N	1999	2000
cm	kg N ha ⁻¹	-----	# -----
2.5	0	1	4
	80	2	4
	160	2	4
5.1	0	2	5
	80	2	5
	160	2	5
7.6	0	3	7
	80	3	7
	160	3	7

Table 3: Harvest period designations for harvest cutting dates in 1999 and 2000.

Havrest period	Year	
	1999	2000
1	Beginning of Season to May 31	Beginning of Season to May 31
2	June 1 to August 31	June 1 to July 31
3	September 1 to end of season	August 1 to end of season

Table 4: Root sampling dates for 1999 and 2000.

Year			
1999		2000	
Month/day	Julian date	Month/day	Julian date
Apr 15	105		
May 27	147	May 30	151
Sept 22	265	Aug 28	241
Oct 25	298	Oct 13	287

Note: The eighth and final root sample was taken April 9, 2001. The data is not included in the results presented.

Pasture Production and Quality

Using a garden drop fertilizer spreader, NH_4NO_3 fertilizer was applied to each plot. Fertilizer was applied first to the center of the plot. This ensured proper coverage of the harvest and destructive sampling areas. The sides and ends were then fertilized.

Herbage in the 0.5 x 2m (1.75 x 6.5 ft) herbage sampling area was cut and collected. A rotary lawn mower with a bag attachment was used to harvest herbage. Mower height was adjusted according to cutting height treatment. The rest of the plot was then cut and herbage removed and collected.

Herbage accumulation

Herbage from the sampling area was collected and weighed on a scale in the field. Sub-samples were taken, weighed and dried for 48 h at 65 C. Herbage accumulation was as the herbage weight multiplied by the dry matter percentage of the sub-sample. These dried sub-samples were ground through a 1 mm mesh screen in a Wiley Mill and placed in plastic bags for fiber and N analysis. For successive harvests, herbage on each plot was allowed to grow to 12.7 cm and then was cut according to its designated treatment. Harvesting and sampling methods were repeated.

Total Kjeldahl nitrogen

Using the macro-Kjeldahl method, the dried and ground samples were analyzed for N content (Horneck and Miller, 1998; Jones, 1991). Duplicates of each sample were placed in a Tecator digestion system 1015 digester. They were then analyzed on a Tecator Kjeltac Auto 1030 Analyzer. Crude protein was calculated on a dry weight basis as the total Kjeldahl N multiplied by 6.25. Crude protein yield was calculated by:

$$\text{CP yield} = (\text{Herbage accumulation} \times \text{CP concentrations})/100$$

Neutral detergent fiber and acid detergent fiber

Neutral detergent fiber and ADF content were determined using the Ankom procedure (Ankom Technology, 1997a and b). Approximately 0.50 g of dried sub-sample were placed in Ankom Technology F57 filter bags and sealed (Ankom Technology, 1997a). Samples were digested in the sealed filter bags in an Ankom 200 Fiber Analyzer. They were then dried for 48 h at 65 C and reweighed. Percent NDF on a dry matter basis was determined using the following equation:

$$\% \text{ NDF} = [100 (W_3 - (W_1 \times C))]/ W_2$$

where W_1 = bag tare weight, W_2 = sample weight, expressed on a dry matter basis, W_3 = final bag and NDF fiber weight, and C = blank bag correction (final oven-dried weight/original blank bag weight).

The Ankom procedure was used for determining percent ADF (Ankom Technology, 1997b). The material left from NDF digestion were again digested in an Ankom 200 Fiber Analyzer, dried for 48 h at 65 C and reweighed. The following equation was used to determine percent ADF on a dry matter basis:

$$\% \text{ ADF} = [100 (W_4 - (W_1 \times C)) / W_2]$$

where W_1 = bag tare weight, W_2 = sample weight, expressed on a dry matter basis, W_4 = final bag and ADF fiber weight, and C = blank bag correction (final oven-dried weight/original blank bag weight).

Pasture Root Growth

Using a golf hole cutter, one 10 cm (4.0 in) diameter, 20.3 cm (8 in) deep soil/root core was extracted from each plot in mid-April, late May, late September, and late October during 1999. In 2000, samples were removed from all plots three times: late May, late August, and mid-October (Table 4). The samples were taken from their assigned areas in the destructive portion of each plot (Fig. 1), placed in plastic bags and refrigerated. The root cores were divided into three sections according to the following depths: 0.0 - 5.1 cm (0.0 – 2.0 in), 5.1 - 10.2 cm (2.0 – 4.0 in), and 10.2 - 20.3 cm (4.0 – 8.0 in). Roots in each section were washed free of soil through a mesh screen, placed in plastic bags and frozen until they were analyzed.

Root volume

The roots were thawed and placed on absorbent paper towels to remove excess water. The wet weight was taken, and the root volume was determined by water displacement.

Root length

Root length was measured on samples from plots in blocks 3 and 4 that received the following treatment combinations: 2.5 cm cut/no N, 2.5 cm cut/high N, 7.6 cm cut/no N and 7.6 cm cut/high N. Root length of each section was determined using ROOTEDGE (Kaspar and Ewing, 1997). Roots were separated and placed on a Hewlett-Packard desktop scanner. Using a microcomputer, the roots were scanned and a black and a white computer TIFF image file for each sample was created. Two or three images per sample were made if the size prevented the entire sample from being placed on the scanner. Difficulties were encountered separating the fine grass roots. Although time and care were taken the samples did contain overlapping roots as well as several roots in a group. This was especially a problem for the 0.0 - 5.1 cm root sampling depth. The computer images were then analyzed using the DOS-based ROOTEDGE program to determine the overall length of the root sample. This program measures the length (cm) of roots using the edge chord algorithm (Ewing and Kaspar, 1995). For samples requiring more than one TIFF image, lengths determined for each image of the sample were summed.

Root dry weight

After the root lengths of the samples were determined, samples were dried for approximately 48 h in a 65 C oven, and weighed to determined dry root mass.

Soil Nitrate

Prior to each N application, an anion transfer membrane strip was placed in each plot to determine soil nitrate levels (NO_3^-). The membranes were prepared for placement into the soil. They were washed with deionized distilled water, agitated in 0.5 M HCl, rewashed with deionized distilled water, saturated with 1 M NaCl for 2 h and rinsed three times with deionized distilled water (Ionics, 1990; Collins and Allinson, 1999). A string was attached to one end to ease removal from the soil. At an approximate depth of 15 cm, one prepared membrane, 2.5 x 6.25 cm, was placed in each plot, in the 15 x 40 cm area for the successive root core. Using a shovel with a flat blade, a wedge (approximately 1-in deep) was created and removed. A 15 cm deep slit was made with the shovel, any extraneous herbage was removed and an anion transfer membrane was placed in the slit. The soil wedge was replaced and tamped down to ensure adequate soil-membrane contact.

Due low rainfall the anion transfer membranes were allowed to remain in the soil for 20 d in June 1999, 17 d in September 1999 and 22 d in September 2000. The anion transfer membranes remained in the plots for 11 d in June 2000. The membranes were removed from the soil by dislodging the soil wedge and gently pulling on the string attached to the end of the anion transfer membrane. Once removed, the anion transfer membranes were rinsed with deionized distilled water to remove soil. In 1999, the membranes were combined by block and N treatment, and placed in 30 ml of a 1 M NaCl solution. However, in 2000 anion transfer membranes from each plot were kept separate and analyzed individually.

The NO_3^- was extracted from the anion transfer membranes by agitating the membranes in the 1 M NaCl solution for 2 h on a wrist action shaker. Anion transfer membranes were then removed from the solutions and discarded. Extract solutions were filtered through Whatman 42 filter paper. Nitrate concentration was determined colorimetrically by low flow injection analysis on a Lachat QuikChem FIA+8000. Nitrate concentrations were reported as mg N l^{-1} . Concentrations were converted to $\mu\text{g N cm}^{-2} \text{ d}^{-1}$.

Climate Report

Daily minimum and maximum temperatures and daily precipitation were obtained from the Morgantown Municipal airport for the period March to October of 1999 and 2000.

Statistical Analysis

Treatment effects on herbage accumulation, forage quality, CP yield, root growth parameters and soil NO_3^- were analyzed using the general linear model procedure (SAS, 1990). Differences between N and cutting treatments, harvest periods and harvest dates were compared using orthogonal contrasts. No N was compared with the medium and high N, and medium N was compared to high N. The 2.5 cm cutting management was compared with the 5.1 and 7.6 cm cutting managements, and the 5.1 cm cutting management was compared with the 7.6 cm cutting management. For herbage accumulation and quality, harvest period 1 was compared with harvest periods 2 and 3, and harvest period 2 was compared with harvest period 3. There were six orthogonal

comparisons for root sampling dates. The pre-treatment root samples (April 15, 1999) were compared with the post-treatment root samples (May 27, 1999 to October 13, 1999). The 1999 season was compared with 2000. September 22, 1999 was compared with the May 27 and October 25, 1999, and May 27, 1999 was compared with October 25, 1999. August 28, 2000 was compared with May 30 and October 13, 2000, and May 30, 2000 was compared with October 13, 2000.

RESULTS

Pasture Production and Quality

Herbage accumulation

Annual herbage accumulation was not affected by any of the N treatments (Table 5 and Appendix Table A). However, cutting management did affect the annual herbage accumulation ($P < 0.0001$). Pasture cut to 2.5 cm cut produced much more herbage than pasture cut to 5.1 or 7.6 cm. The significant interaction ($P < 0.05$) found between cutting management and year did not change the main effect results (Fig. 2).

Herbage accumulation in 2000 was much greater than in 1999 ($P < 0.0001$). In 2000, 4009 kg ha⁻¹ was harvest compared to 1962 kg ha⁻¹ harvested in 1999. An interaction ($P < 0.01$) between N level, cutting management and year was also found.

Figure 2: Effect of year and cutting management on annual herbage accumulation. The interaction between year and cutting management was significant at $P < 0.05$. Year was significant at $P < 0.0001$, and cutting management was significant at $P < 0.0001$.

Table 5: Effect of N treatment, cutting management and year on annual herbage accumulation and CP yield.

Treatment	Herbage accumulation	CP yield
	-----kg ha ⁻¹ yr ⁻¹ -----	
N application		
none	2765	500
medium	3122	575
high	3069	569
Significance [†]	NS	NS
none vs. medium and high	NS	NS
medium vs. high	NS	NS
Cutting management		
2.5 cm	3740	648
5.1 cm	2560	470
7.6 cm	2655	526
Significance	***	**
2.5 cm vs. 5.1 and 7.6 cm	***	***
5.1 cm vs. 7.6 cm	NS	NS
Year		
1999	1962	343
2000	4009	753
Significance	***	***

[†]Significance of F test. NS, non-significant *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: For herbage accumulation:

-Cutting management x year was significant at P < 0.05.

-N x cutting management was significant for P < 0.01.

For CP yield:

-N x year was significant at P < 0.05.

-N x cutting management x year was significant for P < 0.01.

Crude protein concentration

Although N application was not found to significantly affect CP concentration in forage, a significant ($P < 0.05$) difference was found between the CP in plants treated with no N compared to those treated with the medium and high N levels. At the no N treatment level, plants had an average of 17.6% CP. However, in plants to which medium and high levels of N were applied, CP levels were 18.5% and 18.7%, respectively (Table 6 and Appendix Table B). A significant ($P < 0.01$) interaction between N treatment and year showed that the difference between N treatments was only found in 1999 (Fig. 3). An interaction ($P < 0.01$) between N treatment, cutting management and year was found (Fig. 4). Crude protein concentration of herbage was higher for all N treatments and cutting managements in 2000, compared to 1999, except the medium N and 2.5 cm cut and the high N and 2.5 cm cut.

In 2000, a significant ($P < 0.05$) interaction between N application and harvest period was found for CP concentration (Fig. 5). Crude protein was highest in harvest period 3 for plants that were treated with the no N and the high N level. Plants treated with the medium level of N had the greatest CP in period 2. For plants at each level of N, the lowest CP was found when they were harvested in period 1. An N, cutting management and harvest period interaction was also found ($P < 0.05$) in 2000.

Cutting management significantly ($P < 0.01$) affected the CP concentration in herbage (Table 6 and Appendix Table B). Crude protein of forage cut at 2.5 cm was significantly less ($P < 0.01$) than forage cut at 5.1 and 7.6 cm. Also, the herbage cut to 5.1 cm cutting treatment had lower CP concentrations ($P < 0.05$) than the herbage cut to

Table 6: Effect of N treatment, cutting management, harvest period and year on CP, NDF and ADF concentrations in herbage.

Treatment	CP	NDF	ADF
	-----%-----		
N application			
none	17.6	56.5	28.4
medium	18.5	56.8	28.2
high	18.7	56.6	27.3
Significance [†]	NS	NS	NS
none vs. medium and high	*	NS	NS
medium vs. high	NS	NS	NS
Cutting management			
2.5 cm	17.3	57.8	30.0
5.1 cm	18.2	57.3	27.8
7.6 cm	19.4	54.7	26.2
Significance	**	***	***
2.5 cm vs. 5.1 and 7.6 cm	**	***	***
5.1 cm vs. 7.6 cm	*	**	**
Harvest period¹			
1	17.6	52.0	24.2
2	19.8	57.2	31.2
3	20.5	55.3	34.0
Significance	***	***	***
period 1 vs. periods 2 and 3	***	***	***
period 2 vs. period 3	**	***	***
Year			
1999	17.4	58.4	26.8
2000	19.2	54.8	29.1
Significance	***	***	***

¹Harvest period was analyzed for 2000 only.

[†]Significance of F test. NS, non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: For CP:

- N x harvest period and N x cutting management x harvest period were significant at P < 0.05.
- N x year, cutting management x year and N x cutting management x year were significant at P < 0.01.
- Cutting management x harvest period was significant at P < 0.0001.

For NDF:

- Cutting management x year was significant at P < 0.01.
- Cutting management x harvest period and N x cutting management x harvest period was significant at P < 0.0001.

For ADF:

- N x year and cutting management x year interactions were significant at P < 0.01.
- Cutting management x harvest period interaction was significant for P < 0.0001.

Figure 3: Effect of year and N treatment on CP concentration in herbage. Nitrogen was applied two times each year. The interaction of year and N treatment was significant at $P < 0.01$. Year was significant at $P < 0.0001$, and N was NS. The orthogonal comparison of no N vs. medium and high N was significant at $P < 0.05$.

Figure 4: Effect of N treatment, cutting management and year on CP concentration in herbage. The interaction between N treatment, cutting management and year was significant at $P < 0.05$. Nitrogen was NS, but the orthogonal comparison of no N vs. medium and high N was significant at $P < 0.05$. Cutting management was significant at $P < 0.01$, and year was significant at $P < 0.0001$.

Figure 5: Effect of harvest period and N treatment on CP concentration in herbage harvested in 2000. The interaction between harvest period and N treatment was significant at $P < 0.05$. Harvest period was significant at $P < 0.0001$. Nitrogen was NS, but the orthogonal comparison of no N vs. medium and high N was significant at $P < 0.05$.

7.6 cm. Crude protein values for the herbage cut at the 2.5, 5.1 and 7.6 cm levels were 17.3%, 18.2% and 19.4%, respectively.

A significant ($P < 0.01$) interaction was found between cutting management and year (Fig. 6). Herbage harvested in 2000 had the highest CP. However, the CP values of herbage cut to 2.5 cm were very similar each year. An interaction between cutting management and harvest period was also found ($P < 0.0001$) in 2000 (Fig. 7). Crude protein was greatest for herbage harvested at the 5.1 and 7.6 cm management in period 3. Herbage harvested at the 7.6 cm management contained the highest CP in harvest period 2. The lowest CP concentrations for each cutting management were found in herbage harvested in period 1.

Figure 6: Effect of year and cutting management on CP concentration in herbage. The interaction between year and cutting management was significant at $P < 0.01$. Year was significant at $P < 0.0001$, and cutting management was significant at $P < 0.01$.

Figure 7: Effect of harvest period and cutting management on CP concentration in herbage harvested in 2000. The interaction was significant at $P < 0.0001$. Harvest period was significant at $P < 0.0001$, and cutting management was significant at $P < 0.01$.

In 2000, the effect of harvest period on the concentration of CP in the forage was highly significant ($P < 0.0001$) (Table 6 and Appendix Table C). Herbage harvested in period 1 had an average CP content of 17.6%. The orthogonal contrast showed that this was less ($P < 0.0001$) than the CP concentration of herbage harvested in periods 2 (19.8%) and 3 (20.5%). In addition, the CP content of herbage harvested in period 2 was ($P < 0.01$) lower than herbage harvested in period 3.

Crude protein concentration of herbage was significantly ($P < 0.0001$) affected by year (Table 6 and Appendix Table B). Herbage harvested in 2000 had a CP content of 19.2%, while herbage harvested in 1999 had a CP content of only 17.4%.

Crude protein yield

The amount of CP harvested was not significantly affected by N treatments (Table 5 and Appendix Table D). However, an interaction ($P < 0.05$) between N and year was found (Fig. 8), which showed that CP yield of the no N treatment was less than that of the other two N treatments in 1999. A significant ($P < 0.01$) interaction between N, cutting management and year was also found.

Cutting management significantly ($P < 0.01$) affected the amount of CP harvested (Table 5 and Appendix Table D). The 2.5 cm cutting management resulted in significantly ($P < 0.0001$) greater CP harvested compared to the 5.1 and 7.6 cm cutting managements. The average amounts of CP removed for the 2.5, 5.1 and 7.6 cm cutting levels were 648 kg ha^{-1} , 470 kg ha^{-1} and 526 kg ha^{-1} , respectively.

Figure 8: Effect of year and N treatments on CP yield. The interaction between year and N treatments was significant at $P < 0.05$. Year was significant at $P < 0.0001$, and cutting management was significant at $P < 0.01$.

In 2000, harvest period did significantly affected CP yields ($P < 0.01$) (Table 5 and Appendix Table E). In addition the amount of CP harvested was greater in period 1 than in periods 2 and 3 when herbage was cut to 5.1 or 7.6 cm. Herbage had the greatest CP yield in harvest period 2, being much lower in period 1.

Year was found to significantly ($P < 0.0001$) affect the amount of CP harvested. The CP harvested in 2000 ($753 \text{ kg ha}^{-1} \text{ yr}^{-1}$) was more than double the amount harvested in 1999 ($343 \text{ kg ha}^{-1} \text{ yr}^{-1}$) (Table 5 and Appendix Table D).

Neutral detergent fiber

Nitrogen treatments did not significantly affect NDF concentration in the forage harvested (Table 6 and Appendix Table F). There was an interaction ($P < 0.0001$) between N, cutting management and harvest period, but only in 2000.

Cutting management significantly ($P < 0.0001$) affected the concentration of NDF in the harvested herbage (Table 6 and Appendix Table F). Herbage mowed to a height of 2.5 cm had a higher ($P < 0.0001$) concentration of NDF than herbage mowed to 5.1 and 7.6 cm. Also, herbage cut to 5.1 cm had more ($P < 0.01$) NDF than the 7.6 cm treatment. The average percent NDF for the 2.5, 5.1 and 7.6 cm cutting treatments were: 57.8%, 57.3% and 54.7%, respectively.

In 2000, an interaction ($P < 0.0001$) between cutting management and harvest period was found (Fig. 9). The NDF concentration of herbage cut to 5.1 or 7.6 cm was greatest in harvest period 2; however, for the 2.5 cutting management NDF concentration was greatest for herbage harvested in period 3. There was also an interaction ($P < 0.01$) between cutting management and year (Fig. 10). Highest NDF concentration in 1999 was for herbage cut to 5.1, but in 2000 herbage cut to 7.6 cm had the highest NDF concentration.

In 2000, herbage harvested in period 1 had lower NDF concentrations ($P < 0.0001$) than herbage harvested in periods 2 and 3 (Table 6 and Appendix Table G). The NDF content of herbage harvested in period 2 was greater ($P < 0.05$) than herbage harvested in period 3. Year also significantly ($P < 0.0001$) affected the NDF in the harvested herbage (Table 6). Herbage harvested in 1999 had higher NDF concentrations (58.4%) than herbage harvested in 2000 (54.8%).

Figure 9: Effect of harvest period and cutting management on NDF concentration in herbage harvested in 2000. The interaction between harvest period and cutting management was significant at $P < 0.0001$. Harvest period and cutting management were significant at $P < 0.0001$.

Figure 10: Effect of year and cutting management on NDF concentration in herbage. The interaction between year and cutting management was significant at $P < 0.01$. Year and cutting management were significant at $P < 0.0001$.

Acid detergent fiber

As with NDF, N treatments did not affect the ADF concentration of herbage (Table 6 and Appendix Table F). However, the significant N treatment and year interaction ($P < 0.01$) (Fig. 11) indicated that N treatment reduced ADF concentration in 1999, but not in 2000. Also in 2000, there was an N treatment, cutting management and harvest period interaction.

Cutting management significantly ($P < 0.0001$) affected the concentration of ADF in the herbage that was harvested (Table 6 and Appendix Table F). Herbage mowed to a height of 2.5 cm had a greater ($P < 0.0001$) ADF content than herbage mowed to 5.1 or 7.6 cm. Also, the herbage cut to 5.1 cm had higher ($P < 0.01$) ADF concentrations than herbage cut to 7.6 cm. The average percent NDF for the 2.5, 5.1 and 7.6 cm cutting treatments were: 30.0%, 27.8% and 26.2%, respectively. In 2000, herbage harvested at each cutting level had higher ADF percentages than in year 1999, although a significant ($P < 0.01$) interaction between cutting management and year was found (Fig. 12).

Harvest period significantly ($P < 0.0001$) affected the ADF content in herbage in 2000 (Table 6 and Appendix Table G). Herbage harvested in period 1 had lower ADF (24.2%) than herbage harvested in periods 2 (31.2%) and 3 (34.0%). Also, ADF concentrations of herbage harvested in period 2 were lower ($P < 0.0001$) than period 3. Differences between periods could only be analyzed for the year 2000. A cutting management and harvest period interaction was found ($P < 0.0001$) (Fig. 13) in 2000. Herbage ADF concentration increased during 2000 at each period in the order 7.6, 5.1 and 2.5 cm cutting managements. However, the differences were not all significant.

Herbage harvested in 2000 had significantly ($P < 0.0001$) greater ADF concentration (29.1%) than herbage harvested in 1999 (26.8%).

Figure 11: Effect of year and N treatment on ADF concentration herbage. The interaction between year and N treatment was significant at $P < 0.01$. Year was significant at $P < 0.0001$, and N was NS.

Figure 12: Effect of year and cutting management on ADF concentration herbage. The interaction between year and cutting management was significant at $P < 0.01$. Year and cutting management were significant at $P < 0.0001$.

Figure 13: Effect of harvest period and cutting management on ADF concentration in herbage harvested in 2000. The interaction between harvest period and cutting management was significant at $P < 0.0001$. Harvest period and cutting management were significant at $P < 0.0001$.

Pasture Root Growth

Root volume

Since root volume in the top sampling depth was about 4 times greater than at the lower sampling depths, these results will be emphasized. Roots at the 0.0 – 5.1 cm depth had much greater ($P < 0.0001$) root volume than the other depths (Table 7). A significant difference ($P < 0.0001$) was also found between the 5.1 – 10.2 cm and 10.2 – 20.3 cm depths. Root volumes in the 5.1 – 10.2 cm depth were greater compared to the 10.2 – 20.3 cm depth. There were interactions between depth of sampling and other sources of variation.

Root volume was significantly higher ($P < 0.01$) at the medium and high N application levels, as compared to no N (Table 7 and Appendix Table H). However, this

was only true for the 0.0 – 5.1 cm sample depth, as evidenced by the N and depth interaction ($P < 0.01$) (Fig. 14).

When plants were cut to 2.5 cm, root volume at the 0.0 – 5.1 cm depth was significantly lower ($P < 0.01$) than those cut to 5.1 or 7.6 cm (Table 7 and Appendix Table H). Cutting management did not significantly affect root volume in the lower sampling depths, resulting in a cutting management and sampling depth interaction ($P < 0.05$) (Fig. 15).

Sampling date greatly affected root volume ($P < 0.0001$) (Table 7 and Appendix Table H). At each of the three cutting managements, root volume at the 0.0 – 5.1 cm depth was greatest for roots sampled on May 30, 2000. Compared to the root volumes in 2000, the root volumes for roots in 1999 were much lower ($P < 0.0001$) at the 0.0 – 5.1 cm depth. Roots sampled on April 15, 1999, the pre-treatment sample, had significantly less root volume at the 0.0 – 5.1 cm ($P < 0.05$) and 10.2 – 20.3 cm ($P < 0.01$) depths than the post-treatment samples. Roots at the 10.2 – 20.3 cm depth, sampled on September 22, 1999 compared to those sampled on May 27 and October 25, 1999 reveal root volumes at the latter two dates that were significantly greater ($P < 0.01$). Also, at the 2 lower sampling depths, roots sampled on October 25, 1999 had a much lower ($P < 0.0001$) volume compared to those sampled on May 27, 1999. May 30, 2000 and October 13, 2000 samples had significantly ($P < 0.05$) higher root volumes than the August 28, 2000 samples, but only at the 10.2 – 20.3 cm depth. Also, roots at all three depths had significantly ($P < 0.0001$) lower volume on October 13, 2000 compared to May 30, 2000.

Figure 14: Effect of depth and N application on root volume. The interaction between depth and N treatment was significant at $P < 0.01$. Depth was significant at $P < 0.0001$, and N was significant at $P < 0.05$.

Figure 15: Effect of depth and cutting management on root volume. The interaction between depth and cutting management was significant at $P < 0.01$. Depth was significant at $P < 0.0001$, and cutting management was NS.

Table 7: Effect of depth, N treatment, cutting management and sampling date on root volume.

Treatment		Depth		
		0.0 - 5.1 cm	5.1 - 10.2 cm	10.2 - 20.3 cm
N application		cm ³		
	none	18.0	4.2	2.8
	medium	19.8	4.6	3.3
	high	21.1	4.6	3.0
Significance[†]		**	NS	NS
none vs. medium and high		**	NS	NS
medium vs. high		NS	NS	NS
Cutting management				
	2.5 cm	18.1	4.5	3.0
	5.1 cm	20.3	4.5	3.2
	7.6 cm	20.4	4.4	2.8
Significance		*	NS	NS
2.5 cm s. 5.1 and 7.6 cm		**	NS	NS
5.1 cm vs. 7.6 cm		NS	NS	NS
Date				
	April 15, 1999	17.3	3.9	2.2
	May 27, 1999	18.5	5.7	4.6
	September 22, 1999	17.9	4.6	2.6
	October 25, 1999	17.3	4.2	2.6
	May 30, 2000	25.6	6.1	4.0
	August 28, 2000	21.2	3.7	2.5
	October 13, 2000	19.5	3.0	2.5
Significance		***	***	***
April 15, 1999 vs. May 27, 1999 to October 13, 2000		*	NS	**
1999 vs 2000		***	NS	NS
September 22, 1999 vs. May 27 and October 25, 1999		NS	NS	**
May 27, 1999 vs. October 25, 1999		NS	**	***
August 28, 2000 vs. May 30 and October 13, 2000		NS	NS	*
May 30, 2000 vs. October 13, 2000		***	***	**

[†]Significance of F test. NS, non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: There were no significant interactions.

Root dry weight

Nitrogen significantly ($P < 0.01$) affected root dry weight, but only at the 0.0 – 5.1 cm depth (Table 8, Appendix Table I and Fig. 16). At this depth, roots treated with the medium and high N had significantly ($P < 0.01$) higher root dry weights compared to roots receiving no N. No significant differences in root dry weight were found between the medium and high N treatments.

Cutting managements did not significantly affect root dry weights. However, a cutting management and depth interaction was present ($P < 0.05$) (Fig. 17). At the 0.0 – 5.1 cm depth significant ($P < 0.05$) differences in root dry weights were found between herbage cut to 2.5 cm and herbage cut to 5.1 or 7.6 cm. Plants that were mowed to the 2.5 cm cutting level had less root dry weight in the top sampling depth than plants mowed to the other heights.

The date on which roots were sampled had a significant effect on root dry weight ($P < 0.0001$) (Table 8 and Appendix Table I). A sampling date and depth interaction was also present ($P < 0.0001$). In the 0.0 – 5.1 cm depth, root dry matter in the pre-treatment sample, April 15, 1999, was significantly ($P < 0.0001$) lower than in the post-treatment samples. Also at the upper root depth, the 1999 samples had significantly ($P < 0.01$) lower root dry weights compared to 2000.

Roots in the 5.1 – 10.2 cm and 10.2 – 20.3 cm depths had greater ($P < 0.0001$) dry weights in the May 27, 1999 samples compared to the October 25, 1999 samples. However, at the 0.0 – 5.1 cm depth, root dry weight was less ($P < 0.05$) for this comparison. Also, in 2000, roots in all depths had significantly ($P < 0.01$) more root dry weight when sampled on May 30, 2000 than on October 13, 2000.

Table 8: Effect of depth, N treatment, cutting management and sampling date on root dry weight.

Treatment		Depth		
		0.0 - 5.1 cm	5.1 - 10.2 cm	10.2 - 20.3 cm
N application		----- g -----		
	none	4.9	1.0	0.6
	medium	5.8	1.1	0.8
	high	6.1	1.1	0.7
Significance		**	NS	NS
none vs. medium and high		**	NS	NS
medium vs. high		NS	NS	NS
Cutting management				
	2.5 cm	5.1	1.0	0.7
	5.1 cm	5.9	1.1	0.8
	7.6 cm	5.7	1.0	0.6
Significance [†]		NS	NS	NS
2.5 cm vs. 5.1 and 7.6 cm		*	NS	NS
5.1 cm vs. 7.6 cm		NS	NS	NS
Date				
	April 15, 1999	4.1	1.0	0.5
	May 27, 1999	4.7	1.6	1.2
	September 22, 1999	5.8	0.9	0.5
	October 25, 1999	5.8	0.8	0.5
	May 30, 2000	7.1	1.3	0.8
	August 28, 2000	6.0	0.8	0.7
	October 13, 2000	5.6	0.8	0.6
Significance		***	***	***
April 15, 1999 vs. May 27, 1999 to October 13, 2000		***	NS	NS
1999 vs 2000		**	NS	NS
September 22, 1999 vs. May 27 and October 25, 1999		NS	*	**
May 27, 1999 vs. October 25, 1999		*	***	***
August 28, 2000 vs. May 30 and October 13, 2000		NS	*	NS
May 30, 2000 vs. October 13, 2000		**	***	NS

[†]Significance of F test. NS, non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: Harvest date x sampling depth interaction was significant for P < 0.0001.

Similar to root volume (Table 7), the depth of sampling also significantly ($P < 0.0001$) affected the root dry weights (Table 8). The roots at the 0.0 – 5.1 cm depth had a much more dry weight (5.6 g) than roots at the 5.1 – 10.2 cm (1.0 g) and 10.2 – 20.3 cm (0.7 g) depths. The difference between the 5.1 – 10.2 cm and the 10.2 – 20.3 cm depths was also significant, but only at $P < 0.01$.

Figure 16: Effect of depth and N treatment on root dry weight. The interaction between depth and N treatments was significant at $P < 0.01$. Depth was significant at $P < 0.0001$, and N was significant at $P < 0.05$.

Figure 17: Effect of depth and cutting management on root dry weight. The interaction between depth and cutting management was significant at $P < 0.05$. Depth was significant at $P < 0.0001$, and cutting management was NS.

Root length

Nitrogen treatments did not significantly affect the root length. Also, no interactions involving N were found. However, cutting management did significantly ($P < 0.05$) affect root length, but only at the 0.0 – 5.1 cm depth (Table 9 and Appendix Table J). At the upper rooting depth, herbage cut to 2.5 cm had less root length (2267 cm) than that cut to 7.6 cm (2755 cm) (Fig. 18).

Sampling date affected root length at each of the rooting depths (Table 9 and Appendix Table J). A sampling date by depth interaction was also present ($P < 0.0001$). The pre-treatment samples, April 15, 1999, had significantly ($P < 0.05$) less root length at the 10.2 – 20.3 cm than the post-treatment samples. Root length for the 1999 samples was much lower ($P < 0.01$) than the 2000 samples, but only at the 0.0 – 5.1 cm depth.

Table 9: Effect of depth, N treatment, cutting management and sampling date on root length

Treatment		Depth		
		0.0 - 5.1 cm	5.1 - 10.2 cm	10.2 - 20.3 cm
N application		-----cm-----		
	none	2358	1008	831
	medium	-----	-----	-----
	high	2665	997	894
Significance [†]		NS	NS	NS
Cutting Management				
	2.5 cm	2267	961	828
	5.1 cm	-----	-----	-----
	7.6 cm	2755	1045	897
Significance		*	NS	NS
Date				
	April 15, 1999	2188	854	632
	May 27, 1999	2265	1091	990
	September 22, 1999	2386	1061	839
	October 25, 1999	1534	817	651
	May 30, 2000	4153	1448	1273
	August 28, 2000	2412	843	737
	October 13, 2000	2640	907	917
Significance		***	**	**
April 15, 1999 vs. May 27, 1999 to October 13, 2000		NS	NS	*
1999 vs 2000		**	NS	NS
September 22, 1999 vs. May 27 and October 25, 1999		NS	NS	NS
May 27, 1999 vs. October 25, 1999		NS	NS	*
August 28, 2000 vs. May 30 and October 13, 2000		**	*	*
May 30, 2000 vs. October 13, 2000		**	**	*

[†]Significance of F test. NS, non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: Harvest date x depth interaction was significant for P < 0.0001.

Figure 18: Effect of depth and cutting management on root length. The interaction between depth and cutting management was significant at $P < 0.05$. Depth was significant at $P < 0.0001$, and cutting management was NS.

No significant differences in root length between the two years were found at the other depths. Root lengths at the 10.2 – 20.3 cm depth of the samples taken on May 27, 1999 were greater than those taken on October 25, 1999 at the same depth. Also, at each depth, samples taken on August 28, 2000 had less root length than May 30, 2000 and October 13, 2000. Differences between the latter two dates were also found at each of the three rooting depths. The May 30 samples had significantly greater root length than the October 13 samples.

Depth of sampling had a significant ($P < 0.0001$) effect on root length. Roots at the 0.0 – 5.1 cm depth had a much greater root length than roots at the 5.1 – 10.2 cm and 10.2 – 20.3 cm depths. However, no significant differences in root length were found between the 5.1 – 10.2 cm and 10.2 – 20.3 cm depths.

Soil Nitrate

The 1999 season

Nitrogen treatment significantly ($P < 0.05$) affected soil NO_3^- concentrations. However, these differences were only significant between the no N level compared to the medium and high N levels (Table 10 and Appendix Table K). Average soil NO_3^- concentrations for the low, medium and high N treatments were: 0.43, 0.83 and 1.28 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$, respectively.

Date of N application also affected soil NO_3^- levels. The soil treated in June had significantly ($P < 0.01$) lower NO_3^- levels than the September 1999 application (Table 10 and Appendix Table K). The average soil NO_3^- concentration after the June application was 0.59 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$. However, in September, the average concentration was 1.10 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$.

In 1999, anion transfer membranes in each block were grouped according to N treatments for analysis, and cutting treatment effects could not be determined.

The 2000 season

Nitrogen application was also found to significantly ($P < 0.05$) affect soil NO_3^- concentrations in the 2000 season. Soil with no N added had a significantly ($P < 0.05$) lower soil NO_3^- concentration (1.00 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$) compared to soil to which medium (1.71 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$) and high levels of N were applied (1.87 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$) (Table 10 and Appendix Table K). However, there was no difference between the medium and high N treatments.

The effects of cutting treatment were determined in the 2000 season. The 2.5 cm cutting treatment resulted in significantly ($P < 0.01$) greater soil NO_3^- concentrations

Table 10: Effect of N treatment, cutting management and N application date on soil nitrate concentration in 1999 and 2000.

Treatment	Average NO₃⁻ Concentration	
	1999	2000
N application	---μg N cm ⁻² d ⁻¹ ---	
none	0.43	1.00
medium	0.84	1.71
high	1.28	1.88
Significance [†]	*	*
none vs. medium and high	*	*
medium vs. high	NS	NS
Cutting management		
2.5 cm	-----	2.09
5.1 cm	-----	1.32
7.6 cm	-----	1.18
Significance	-----	*
2.5 cm vs. 5.1 and 7.6 cm	-----	**
5.1 cm vs. 7.6 cm	-----	NS
N application date		
June 3, 1999	0.59	-----
September 10, 1999	1.10	-----
June 2, 2000	-----	2.13
August 30, 2000	-----	0.93

[†]Significance of F test. NS, non-significant; *, P < 0.05;

** P < 0.01; *** P < 0.0001; NS = not significant.

Note: N x cutting management x date interaction in 2000 was significant at P < 0.05.

Figure 19: Effect of N treatment, cutting management and sampling date on soil NO_3^- concentration in 2000. The interaction between N treatment, cutting management and sampling date was significant at $P < 0.05$. Nitrogen and cutting management were significant at $P < 0.05$. Sampling date was significant at $P < 0.0001$.

than did cutting treatments 5.1 and 7.6 cm (Table 10 and Appendix Table K). The average soil NO_3^- concentrations of the 2.5, 5.1 and 7.6 cm cutting managements were: 2.09, 1.32 and 1.18 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$. No significant differences were found between the 5.1 and 7.6 cm cutting managements.

Date significantly ($P < 0.0001$) affected soil NO_3^- concentrations in 2000. However, unlike the 1999 season, the June 2000 date resulted in significantly higher soil NO_3^- concentrations than August 2000. The average soil NO_3^- concentration for June was 2.13 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$; the average for August was 0.93 $\mu\text{g N cm}^{-2} \text{ day}^{-1}$ (Table 10 and Appendix Table K).

An interaction ($P < 0.05$) between N, cutting management and date was present (Fig. 19). All soil NO_3^- concentrations at each level of N and for all three cutting managements, except the high N and 2.5 cm cut, were highest in June 2000.

Soil Samples

The pH of the soil was significantly ($P < 0.01$) affected by year, being slightly higher in 1999 (6.37) than 2000 (6.29). Year also significantly ($P < 0.0001$) affected available soil phosphorous (P), calcium (Ca) and magnesium (Mg). Available soil P and Mg were greater in 1999. However, available Ca was greater in 2000 (Table 11 and Appendix Table L). Nitrogen and cutting treatments had no significant effects on soil pH, P, Ca and Mg. There were no differences between the sampling depths.

N application level, cutting management or sample year, did not significantly affect the availability of potassium (K) in the soil. However, an interaction between N and year was present ($P < 0.05$). Soil K was greater in 1999 at the no N and the medium N treatments and in 2000 at the high N treatment. An interaction was also present between cutting management and year ($P < 0.01$). There was more available K in 1999 when cutting management was 2.5 cm. In 2000, available K in the soil was greater under the 5.1 and 7.6 cm cutting managements. No differences in available K were found between soil sampling depths.

Table 11: Effect of N treatment, cutting management and year on pH and available P, K, Ca and Mg of soil

Treatment	pH	P	Available			Mg
			K	Ca		
----- kg ha ⁻¹ -----						
N application						
none	6.4	60	259	4211	189	
medium	6.4	61	246	3989	186	
high	6.3	56	247	3871	193	
Significance [†]	NS	NS	NS	NS	NS	
Cutting management						
2.5 cm	6.3	57	249	4116	186	
5.1 cm	6.4	60	248	4005	190	
7.6 cm	6.3	60	254	3946	191	
Significance	NS	NS	NS	NS	NS	
Year						
1999	6.4	62	254	3568	203	
2000	6.3	56	247	4453	176	
Significance	**	***	NS	***	***	

[†]Significance of F test. NS, non-significant, *, P < 0.05; ** P < 0.01; *** P < 0.0001.

Note: For K:

-N x year interaction was significant at P < 0.05.

-Cutting management x year interaction was significant at P < 0.01.

Climate

Average temperatures in 1999 and 2000 were similar from April to October (Fig. 20). Compared to 1999, the average temperature was about 5 C higher in March 2000 and about 4 C less in July 2000. Overall, the 1999 season had much less rainfall than the 2000 season (Fig. 21). From May to August approximately 20 cm more rainfall was recorded in 2000 compared to 1999. However, approximately 5 cm more rainfall was recorded for September 1999 than September 2000. Rainfall was not recorded for October 2000.

Figure 20: Average monthly temperature from March to October 1999 and 2000, as recorded at Morgantown Municipal Airport.

Figure 21: Total monthly rainfall from March to October 1999 and 2000, as recorded at Morgantown Municipal Airport.

DISCUSSION

Pasture Production and Quality

In 1999, rainfall was much lower than in 2000. The highly significant effect of year on plant production and quality is a result of differences in rainfall between 1999 and 2000. The same is true for significant interactions found involving year, N treatment and/or cutting management. Herbage accumulation, CP concentration, CP yield, and ADF concentrations of herbage were all much higher in 2000 compared to 1999. MacKlon, et al., (1996) found that herbage accumulation in pastures decreased 25 to 50% in dry years compared to years with normal rainfall, and changes in soil water affected the N content of the herbage. However, herbage NDF concentration was higher in 1999. This higher ADF concentration may be attributed to the delay of plant maturity caused by the drought stress.

Harvest date (in 1999 and 2000) and harvest period (2000 only) significantly affected CP concentration, CP yield and NDF and ADF concentrations. These effects were probably caused by rainfall, stand maturity, timing of N applications, and/or the frequency of harvest.

Effects of nitrogen fertilizer

Herbage accumulation, CP yield, and NDF and ADF concentrations were not affected by the addition of N fertilizer. Although there was no significant main effect of N on CP concentration, orthogonal comparisons did indicate that CP concentration was lower in herbage receiving no N compared to herbage receiving medium and high N levels.

Response of a grass-legume stand to N fertilization depends on plant species, botanical composition, soil moisture and availability of other necessary nutrients (Baylor, 1974). In terms of herbage accumulation, the yield responses from N fertilization have been shown to be small. In alfalfa-grass mixtures applications of up to 112 kg N ha⁻¹ have resulted in no yield increase to only slight yield increases (Parsons, 1958). Carter and Scholl (1962) found similar results and attributed the slight yield increase to an increase in the percentage of grass in the stand. Generally, a higher grass percentage in the stand will result in a greater yield response to N (Baylor, 1974).

The timing of N applications can be very important. Wedin (1974) states that cool-season grasses fertilized before the cool season starts have a very high N use efficiency. In this experiment N fertilizer was applied in early June, when Kentucky bluegrass has already reached its growth peak, and in late August, before the usual fall increase. Perhaps the lack of response to N was a result of applying N too late in the spring, after the peak of growth. Since rainfall was low following both N fertilization times in 1999 and after the August fertilization in 2000, the plants may have taken up less N. Therefore, they would not have responded well, if at all, to the N application. The low rainfall in 1999 and in late summer of 2000 influenced the production and quality of herbage.

The effect of N fertilization on CP has been shown to parallel the effect of N fertilization on yield (Cosper and Thomas, 1961; Reid and Castle, 1965). Crude protein concentration of forage is dependent not only on N fertilization, but plant species, existing soil fertility and cutting management (Baylor, 1974). In mixed swards, N fertilization will often favor grasses and subsequently decrease the percent of legumes

present. This decrease of legume may also decrease protein concentration of the sward (Baylor, 1974).

The CP concentration increased when plants were fertilized with N, but only in 1999. The below normal rainfall in 1999 caused N to be more concentrated in the herbage. Crude protein is directly related to the N concentration in plants. Nuttall (1980) found that as N fertilization rates increased so did the concentration of N in bromegrass. Therefore, it is reasonable to assume that in this study, the addition of N fertilizer increased the N concentration of the herbage, thus increasing CP concentration.

The N treatment by harvest period interaction showed that CP concentration of herbage for all N treatment levels, except the medium N level, increased as harvest period increased. The cause of this interaction is not clear. The N treatment by harvest date interaction showed that herbage treated with the medium N level had the lowest CP concentrations at every harvest date in 2000, except on June 29, when it had the highest CP concentrations. This was probably a result of the first N fertilization approximately three weeks prior to the harvest.

Crude protein yields were not different between the plants fertilized with N and those that were not fertilized with N. The result is not like that found for the CP concentration in forage. This is probably because herbage yield was not affected by N applications.

Fiber (NDF and ADF) concentration of herbage is usually not strongly affected by N fertilization, as shown by the results of this experiment. The results of several other studies agree. Nitrogen fertilization increased yield and CP, but not plant digestibility or percent fiber (Williams, 1962; Washko, 1963; Hart and Burton, 1965).

Effects of cutting management

Cutting management greatly affected herbage accumulation, CP concentration and yield, and NDF and ADF concentrations. The 2.5 cm cutting management yielded more herbage than the 5.1 and 7.6 cm cutting managements. Frequency of harvest did not seem to be the reason for this difference in herbage accumulation. In 1999, the 2.5 cm treatment was harvested at about the same frequency as the 5.1 and 7.6 cm treatments. In 2000, it was harvested slightly fewer times than the 5.1 and 7.6 cm treatments (Table 2). Therefore, the cutting intensity was the reason for differences in herbage accumulation. However, Bryan et al. (2000) found that when defoliation occurred with high frequency and intensity growth rate was reduced. The authors concluded that herbage accumulation was greatest with frequent defoliation of approximately 50% (low intensity) of the herbage. However, the study did find that amount of rainfall greatly affected herbage production. In a dry year, such as 1999, grazing intensity and frequency did not have much affect on herbage growth.

Crude protein concentration, which improves animal performance, was highest in herbage harvested to 7.6 cm and lowest in herbage harvested to 2.5 cm. Mortimer and Ahlgren (1936) found similar results. They found that increased cutting intensity decreased the CP concentration of Kentucky bluegrass. Nitrogen concentration is usually greatest in leaf blades, lower in stems and even lower in roots (Vance and Heichel, 1981). Also, the allocation of N is dependent on light and photosynthesis. Leaves on the upper part of the plant receive more light and have higher N concentrations than leaves lower in the canopy (Field, 1983; Hirose and Werger, 1987; Pons et al., 1989). As the cutting height gets lower, the amount of herbage harvested lower on the plant increases. Since

the herbage harvested at the 7.6 cm cutting management contained mostly the upper leaves, the N and CP concentrations were higher than at the 5.1 and 2.5 cm cutting managements.

The reasons for the interactions found between cutting management and harvest period and N treatment, cutting management and harvest period in 2000 are not clear. Both interactions indicate that for the 2.5 cm cutting management herbage CP concentration was lowest in harvest period three. This could be the result of the lower rainfall in that period. Also, there was a six-week span between the 2.5 cm harvests of herbage in periods 2 and 3. This long regrowth period may have lowered the N and CP concentration in the herbage.

Unlike CP concentration of herbage, CP yield was greatest for the 2.5 cm cutting management. Although CP concentration was lower, the amount of herbage harvested at this cutting management compensated for the low CP concentration.

Herbage quality is never constant; therefore, NDF and ADF concentrations are highly dependent on the maturity of the plant and harvest date. As a plant matures the cell wall increases in lignin and fiber. Leaves contain less fiber than stems, and younger leaves have less fiber than older leaves. The results of this experiment show that both NDF and ADF increased with increased harvest intensity. Because all treatments were allowed to grow to 12.7 cm before they were harvested, maturity and age of the leaves at harvest was not a factor. However, the type of plant material harvested varied by cutting management. The plant material harvested under the 7.6 cm cutting management was primarily the younger leaves of the sward. Since it has been established that younger leaves have lower fiber concentrations, the NDF and ADF concentrations found in this

herbage would also be lower. However, as cutting management intensity increased, the age, NDF and ADF concentrations of the harvested herbage also increased.

Interactions between cutting management and harvest period were found for NDF and ADF. In harvest period 1, there was little difference between the fiber content of the herbage cut at different heights. Compared to the 5.1 and 7.6 cm cutting managements, the herbage harvested at the 2.5 cm cutting management had slightly to much higher concentrations of both NDF and ADF in harvest period 3. In 2000, the sward cut to the 2.5 cm was only harvested once in period 2 and once in period 3. Before these harvests, approximately 4 to 6 weeks elapsed. During this time the herbage continued to mature. Therefore, when the herbage treated with the 2.5 cm cutting management was finally harvested, the plant material contained much more fiber.

For NDF, an interaction between N treatment, cutting management and harvest period was found in 2000. The NDF concentration was greatest in period 2 for herbage at all treatments, except the no N/2.5 cm and medium N/2.5 cm treatments. The latter two treatment combinations resulted in the highest herbage NDF in harvest period 3. Because the herbage cut to 2.5 cm was not harvested in period 3 after N fertilizer was applied, it is not clear why this interaction occurred. The reason is probably related to the cutting management by harvest period interaction mentioned in the previous paragraph.

Pasture Root Growth

Root volume, dry weight and length were about 4 times greater in the upper sampling depth (0.0 – 5.1 cm) compared to the lower two depths (5.1 – 10.2 and 10.2 – 20.3 cm). These results confirm the findings in the literature cited that compared to

similar grasses, Kentucky bluegrass has a rather shallow root system. Generally it has a larger root mass and greater root development in the top 7.6 cm (3 in) of soil, and root mass and development have been shown to decrease very quickly at lower rooting depths (Gist and Smith, 1948). Also, because white clover is a shallow-rooted plant, the majority, if not all, of its roots would have been found in the top sampling depth. Therefore, compared to other plants, Kentucky bluegrass and white clover are more able to compete for water and nutrients in the upper 5.1 cm of the soil than at lower depths.

At the 0.0 – 5.1 cm depth the root volume, dry weight and length of Kentucky bluegrass were greater in 2000 compared to 1999. This may be explained by below normal rainfall in 1999 compared to above normal rainfall in 2000. Available water in the upper soil layer was quickly depleted by the large root volume and by evaporation. Regular rainfall would have been needed to maintain root growth.

Root mass varied according to sampling date; however, differences between sampling dates were more pronounced in 2000 than in 1999. At most depths in 1999 and at all depths in 2000, the May root samples had the greatest root volume, dry weight and length. Autumn and spring usually favor root growth and production of Kentucky bluegrass because soil temperatures are lower (Brown, 1943). However, root growth is usually greatest in the spring (Sprague, 1933; Stuckey, 1941). Hanson and Juska (1961) found that the growth of Kentucky bluegrass roots increases in February and March, and this stimulated growth continues through May. Because the greatest root volumes, dry weight and lengths were usually found in the May root samples, the results of the experiment agree with the results of Hanson and Juska.

Effects of nitrogen fertilizer

Medium and high N applications increased root volume and dry weight, however, only in the 0.0 - 5.1 cm rooting depth. These results can be explained by the results of other experiments. Uptake of N is dependent on the concentration of N and the rate of plant growth (Drew, 1975). Generally, plants receiving no N have significantly lower root weights than plants fertilized with N. However, there is usually little difference in root weight between plants receiving low and high N applications as long as the levels of other necessary nutrients are adequate (Benedict and Brown, 1944; Holt and Fisher, 1960; Lorenz and Rogler, 1966). Also, N fertilization in September can often increase the amount of roots produced during the winter months (Hanson and Juska, 1961).

The root volume and dry weight in the top 5.1 cm of soil was much greater than the root volumes and dry weights at the other two depths. This finding may be explained by the rooting characteristics of Kentucky bluegrass and white clover that were mentioned previously. However, it may also be explained by the availability of nutrients. Root development is often altered by the availability of nutrients in the soil profile; more roots are usually found in soil volumes high in necessary nutrients (Drew, 1975). Since the N fertilizer was applied to the soil surface the N concentration was probably highest in the top 5.1 cm of the soil. Therefore, higher root volumes and dry weights were found in the top layer because roots usually proliferate in areas of higher N concentration (Vogt et al., 1993). This may also explain why no differences in root length between N treatments were found in the 5.1 - 10.2 cm and 10.2 - 20.3 cm depths. However, no differences in root length between the no N and high N treatments were found in the top rooting depth either.

Effects of cutting management

Cutting management had a greater effect on root growth at the 0.0 - 5.1 cm depth than at any other depth. The experimental results showed that, at the 0.0 - 5.1 cm depth, root volume, dry weight and length were lower for plants cut to 2.5 cm compared to those cut to 5.1 and 7.6 cm. This effect of defoliation height is similar to that found by Sprague (1933) and Coupland et al. (1960). For a stand of Kentucky bluegrass maintained at a height of 2.2 cm (0.9 in) compared to an uncut stand, Sprague only found differences in the root dry weights in the 0.0 - 2.5 cm depth. An explanation for the effect of cutting management at the top sampling depth may be that severe defoliation reduces the size of the roots and causes less branching to occur in the upper soil layer (Schuster, 1964).

Defoliation generally reduces or stops root growth temporarily, and it has been found to reduce root mass (Thornton and Millard, 1996). However, regrowth of roots is generally rapid. Similarly, soon after 50% or more herbage is removed from C₃ grasses, root growth often ceases (Richards, 1993) and some of the fine roots may senesce. In this experiment because more than 50% of the shoots were removed when the herbage was defoliated to 2.5 cm, root growth probably stopped almost immediately. Therefore, the root volume, dry weight and length would subsequently decrease. However, more than 50% of the shoots were also removed at the 5.1 cm cutting management, and root volume, dry weight and length were not affected as much. Because the 5.1 cm cutting management was not as severe as the 2.5 cm cutting management, the plants may have been able to recover sooner. Therefore, the amount of time during which root growth ceased may have been shorter at the 5.1 cm cutting height. Also, fewer fine roots may have senesced.

Soil Nitrate

Effects of nitrogen fertilizer

In both 1999 and 2000, soil treated with no N had lower soil NO_3^- concentrations than soil treated with the medium and high N levels. It is reasonable to assume that soil NO_3^- levels will be lower in soils to which no N has been added. In related experiments, a strong relationship was found between N application level and NO_3^- desorbed to the ion membrane strips (Pare et al., 1995; Ziadi and Simard, 1996; Collins and Allinson, 1999). The results of this experiment do not totally agree since no differences in soil NO_3^- were found between the medium and high N applications; however, the differences between the medium and high N treatments were close to significant. Because N flow and uptake are dependent on water flow and uptake, the absence of a difference between the medium and high N fertilization levels could be attributed to the lower rainfall in 1999 and part of 2000. When water supply is low soil NO_3^- is "incompletely exploited" (Havill et al., 1977). The differences found between years and N application dates can be attributed to the amount of rainfall received. The concentration of NO_3^- desorbed by the anion transfer membranes appeared to be directly proportional to rainfall. For example, the amount of NO_3^- desorbed to ion transfer membranes was about 2 to 4 times greater for the June 2000 N application compared to any other time. The amount of rainfall received in June 2000 was approximately 11 cm, 5 to 7 cm greater than the amount of rainfall received following other N applications (Fig. 21). Also, rainfall in late summer and fall of 1999 was approximately 4 cm greater than late spring and early to mid-summer; soil NO_3^- concentrations were found to be 2 times greater in September 1999 compared to

June 1999. The results also indicate that soil NO_3^- was better exploited by plant roots when rainfall was higher.

The root growth patterns of Kentucky bluegrass and white clover could have also contributed to soil NO_3^- concentrations. The extent of a root system is a strong indicator of the ability of plants to compete for and absorb NO_3^- from the soil (Andrews and Newman, 1970). The ion transfer membranes were inserted to a depth of approximately 15 cm, but, as previously described, most of the root mass was present in the upper 5.1 cm of the soil. Also, in drier weather temperatures may be higher and more N may volatilize. It is possible that, because N was applied to the soil surface, and the amount of rainfall was generally low, most of the NO_3^- was taken up by the roots in the upper 5.1 cm of soil. Therefore, little NO_3^- was available to leach to the depth of the ion transfer membrane strip.

Effects of cutting management

In 2000, the soil in plots of pasture plants cut to 2.5 cm had higher concentrations of NO_3^- . This too can be attributed to the size of the root system. Because the more severe defoliation decreased root volume, dry weight and length, there were fewer roots to take up large amounts of NO_3^- . Therefore, more NO_3^- was able to leach to the depth of the ion transfer membrane than under pasture cut higher.

There was an interaction found between N level, cutting management and date in 2000. Soil NO_3^- concentrations for each N level and cutting management were highest in June 2000, except the high N and 2.5 cm cut. In June rainfall was higher; therefore, the NO_3^- moved through the soil quickly. Under the high N and 2.5 cm cutting management the smaller root system would have taken up less NO_3^- from the soil. This coupled with

the higher N rate would have made more NO_3^- available to leach through the soil. It is possible that the higher rainfall could have caused much of the NO_3^- to leach through the soil too quickly for it to be taken up by the ion transfer membrane. However, in August and September 2000, the low rainfall would have reduced the mobility of NO_3^- , allowing it to be available to desorb to the ion transfer membrane.

CONCLUSIONS

The results of this experiment indicate that herbage accumulation, CP concentration and yield, and NDF and ADF concentration of herbage were strongly influenced by cutting management. Although the 2.5 cm cutting management resulted in higher herbage production and CP yields, the 7.6 cm cutting management produced herbage of higher quality. However, the amount of herbage harvested at the 2.5 cm cutting management was only 85 kg ha⁻¹ yr⁻¹ greater than that of the 7.6 cm cutting management. Frequency of defoliation did not appear to affect herbage accumulation; however, the intensity of defoliation did directly affected herbage accumulation.

The differences in CP, NDF and ADF concentrations between the 2.5 and 7.6 cm cutting managements varied by only 2 to 3% in both years of the study. The quantity and quality of forage are both very important. When quantity was lower, quality was higher, giving a “balance.” Herbage accumulation was greatest at the 2.5 cm cutting management; however, herbage quality was highest at the 7.6 cm cutting management. Therefore, the results indicate that there may be no differences between cutting managements.

Crude protein concentration was the only herbage characteristic that was increased by the addition of N fertilizer. The concentration of CP in herbage treated with the no, medium and high N was 17.6, 18.5 and 18.7%, respectively. Therefore, the effect of N on CP was not drastic. The small response to the N fertilizer may have been the result of low rainfall. The amount of rainfall also influenced each of the pasture production and quality indicators, giving large differences in results between 1999 and

2000. Quantity and quality of herbage were both greater in 2000, when rainfall was much higher. Approximately 20 cm more rainfall was recorded from May to August 2000 compared to May to August 1999. While N fertilization did increase the quality of the herbage slightly, the effects of the N fertilization did seem to be dependent on the rainfall, and is difficult to predict. In addition, the timing of the N fertilization applications may not have complimented the growth of the pasture well.

Compared to the lower two sampling depths, the root volume, dry weight and length were much greater in the upper 0.0 - 5.1 cm of the soil. This is a result of the root growth patterns of Kentucky bluegrass and white clover. Root volume, dry weight and length were all greater in 2000 than in 1999, due to the below normal rainfall in 1999. Also, root growth was usually highest in the spring.

In the upper 0.0 - 5.1 cm sampling depth, root volume and dry weight were increased by N fertilization. The root growth of patterns of Kentucky bluegrass and white clover naturally result in greater root growth at this depth. Roots generally proliferate in areas of higher nutrient concentrations, and the uptake of N is directly proportional to the amount of roots in a given area. Therefore, since the N fertilizer was applied to the surface of the soil and the amount of roots in the upper 0.0 - 5.1 cm of the soil was large, the roots took up most of the applied N in the upper sampling depth.

Plants cut to 2.5 cm had lower root volume, dry weight and length in the 0.0 – 5.1 cm sampling depth than plants cut to 5.1 or 7.6 cm. More severe defoliation probably caused some roots to die and root growth to stop. The result would be a decrease in root volume, dry weight and length.

In 1999 and 2000, soil treated with the medium and high N fertilization levels had higher soil NO_3^- concentrations than soil to which no N was applied. The roots in the upper 5.1 cm of the soil may have taken up most of the N applied to the surface, leaving little N to leach to the depth of the ion transfer membrane. The low rainfall in 1999 and late summer of 2000 seemed to decrease the amount of NO_3^- that desorbed to the ion transfer membrane. The low rainfall during these periods may be the reason why such large differences were found between 1999 and 2000, and no differences in soil NO_3^- concentration were found between the medium and high N treatments. In addition, the lower abundance of roots in the upper 5.1 cm of the soil could have contributed to the higher soil NO_3^- concentrations found in the soil under plants cut to 2.5 cm. Because severe defoliation decreases root quantity, fewer roots were available to take up NO_3^- from the soil. Therefore, the NO_3^- leached through the soil profile and was available for the desorption to the ion transfer membrane.

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LIST OF APPENDICES

Appendix	Page
A. Analysis of variance of annual herbage accumulation.....	89
B. Analysis of variance for CP content of herbage annual mass.....	90
C. Analysis of variance for CP content of herbage harvested in 2000, meaned within harvest dates.....	91
D. Analysis of variance for CP yield of herbage, summed over harvest dates.....	92
E. Analysis of variance for CP yield means within harvest period in 2000.....	93
F. Analysis of variance for NDF and ADF content of herbage, averaged over harvest dates.....	94
G. Analysis of variance for NDF and ADF content of herbage harvested in 2000, meaned within harvest dates.....	95
H. Analysis of variance for root volume at each sampling depth.....	96
I. Analysis of variance for root dry weight at each sampling depth.....	97
J. Analysis of variance for root length at each sampling depth.....	98
K. Analysis of variance for soil NO ₃ ⁻ for 1999 and 2000.....	99
L. Analysis of variance for soil pH and available soil P, K, Ca and Mg in experimental plots in 1999 and 2000.....	100

Appendix Table A

Analysis of variance for annual herbage accumulation

Source	df	Herbage accumulation
		Analysis of variance partial sums of squares
Block	3	1080417
N	2	1785564
none vs. medium and high	1	1752690
medium vs. high	1	32874
Cutting management	2	20633794***
2.5 cm vs. 5.1 and 7.6 cm	1	20525042***
5.1 cm vs. 7.6 cm	1	108753
N x Cutting management	4	1220244
Error a	24	10958763
Year	1	75433660***
N x year	2	1438977
Cutting management x year	2	2418419*
N x Cutting management x year	4	5800043**
Error b	27	6395350

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table B

Analysis of variance for CP content of herbage annual means

Source	df	CP
		Analysis of variance of partial sums of squares
Block	3	5.70
N	2	16.45
none vs. medium and high	1	15.89*
medium vs. high	1	0.56
Cutting management	2	58.20**
2.5 cm vs. 5.1 and 7.6 cm	1	38.60**
5.1 cm vs. 7.6 cm	1	19.60*
N x Cutting management	4	20.88
Error a	24	67.97
Year	1	60.05***
N x Year	2	19.99**
Cutting management x Year	2	15.63**
N x Cutting management x Year	4	15.80*
Error b	27	32.39

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table C

Analysis of variance for CP content of herbage harvested in 2000, meaned within harvest period

Source	CP	
	df	Analysis of variance of partial sums of squares
Block	3	25.89
N	2	4.40
none vs. medium and high	1	0.09
medium vs. high	1	4.31
Cutting management	2	182.06***
2.5 cm vs. 5.1 and 7.6 cm	1	159.14***
5.1 cm vs. 7.6 cm	1	22.93*
N x Cutting management	4	5.01
Error a	24	72.58
Harvest period	2	164.01***
Harvest period 1 vs. harvest periods 2 and 3	1	156.03***
Harvest period 2 vs. harvest period 3	1	7.98**
N x Harvest period	4	11.56*
Cutting management x Harvest period	4	104.35***
N x Cutting management x Harvest period	8	24.11*
Error b	54	59.04

Note: *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$; Other interaction are NS

Appendix Table D

Analysis of variance for CP yield of herbage, summed over harvest dates

CP yield		
Source	df	Analysis of variance of partial sums of squares
Block	3	62137.40
N	2	84840.85
none vs. medium and high	1	84464.03
medium vs. high	1	376.82
Cutting management	2	401063.21**
2.5 cm vs. 5.1 and 7.6 cm	1	363168.06**
5.1 cm vs. 7.6 cm	1	37895.15
N x Cutting management	4	75901.62
Error a	24	588286.93
Year	1	3032557.07***
N x Year	2	84142.87*
Cutting management x Year	2	27151.16
N x Cutting management x Year	4	259106.31**
Error b	27	296965.53

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table E

Analysis of variance for CP yield means within harvest period in 2000

Source	df	CP yield
		Analysis of variance of partial sums of squares
Block	3	11596.18
N	2	1574.59
none vs. medium and high	1	342.82
medium vs. high	1	1231.77
Cutting management	2	392564.66***
2.5 cm vs. 5.1 and 7.6 cm	1	391964.62***
5.1 cm vs. 7.6 cm	1	600.05
N x Cutting management	4	28066.34
Error a	24	93820.58
Harvest period	2	4658.11
Harvest period 1 vs. harvest periods 2 and 3	1	1265.34
Harvest period 2 vs. harvest period 3	1	3392.76
N x Harvest period	4	5887.44
Cutting management x Harvest period	4	76049.34***
N x Cutting management x Harvest period	8	24453.98
Error b	54	78476.13

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table F**Analysis of variance for NDF and ADF content of herbage, averaged over harvest dates**

Source	df	NDF	ADF
		Analysis of variance of partial sums of squares	Analysis of variance of partial sums of squares
Block	3	69.38**	23.65
N	2	0.85	18.01
none vs. medium and high	1	0.33	8.34
medium vs. high	1	0.52	9.67
Cutting management	2	133.65***	176.36***
2.5 cm vs. 5.1 and 7.6 cm	1	53.27**	144.94***
5.1 cm vs. 7.6 cm	1	80.37**	31.42**
N x Cutting management	4	9.93	27.48
Error a	24	100.36	81.16
Year	1	240.42***	100.32***
N x Year	2	7.96	14.87**
Cutting management x Year	2	25.75**	10.74**
N x Cutting management x Year	4	5.16	6.34
Error b	27	61.74	22.33

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table G

Analysis of variance for NDF and ADF content of herbage harvested in 2000, meaned within harvest period

Source	df	NDF	ADF
		Analysis of variance of partial sums of squares	Analysis of variance of partial sums of squares
Block	3	69.45*	31.72
N	2	22.12	9.59
none vs. medium and high	1	20.42	1.10
medium vs. high	1	1.70	8.49
Cutting management	2	501.94***	810.36***
2.5 cm vs. 5.1 and 7.6 cm	1	473.34***	746.47***
5.1 cm vs. 7.6 cm	1	28.60*	63.89**
N x Cutting management	4	29.60	21.90
Error a	24	125.78	138.72
Harvest period	2	512.25***	1824.74***
Harvest period 1 vs. harvest periods 2 and 3	1	443.86***	1675.37***
Harvest period 2 vs. harvest period 3	1	68.39***	149.37***
N x Harvest period	4	10.05	13.02
Cutting management x Harvest period	4	226.24***	179.40***
N x Cutting management x Harvest period	8	51.23*	50.86*
Error b	54	137.74	157.47

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table H

Analysis of variance for root volume at each sampling depth

Source	Depth					
	0.0 - 5.1 cm		5.1 - 10.2 cm		10.2 - 20.3 cm	
	df	Analysis of variance partial sums of squares	df	Analysis of variance partial sums of squares	df	Analysis of variance partial sums of squares
Block	3	460.77*	3	67.62*	3	42.48*
Nitrogen	2	410.49**	2	9.12	2	8.69
none vs. medium and high	1	338.59**	1	8.91	1	5.11
medium vs. high	1	71.89	1	0.21	1	3.63
Cutting management	2	282.56*	2	0.60	2	5.64
2.5 cm vs. 5.1 and 7.6 cm	1	281.70**	1	0.48	1	0.10
5.1 cm vs. 7.6 cm	1	0.86	1	0.12	1	5.54
Error a	24	838.98	24	154.58	24	112.92
Date/Year	6	1923.11***	6	259.4***	6	176.22***
April 15, 1999 vs. May 27, 1999 to October 13, 2000	1	225.64*	1	11.96	1	21.28**
1999 vs. 2000	1	958.87***	1	18.08	1	4.03
September 22, 1999 vs. May 27 and October 25, 1999	1	0.04	1	2.56	1	23.34**
May 27, 1999 vs. October 25, 1999	1	24.50	1	38.28**	1	70.01***
August 28, 2000 vs. May 30 and October 13, 2000	1	47.32	1	15.84	1	14.78*
May 30, 2000 vs. October 13, 2000	1	666.73***	1	172.67***	1	42.78**
Nitrogen x Date	12	251.20	12	89.80	12	25.30
Cutting management x Date	12	779.74*	12	63.11	12	39.81
Nitrogen x Cutting management x Date	24	742.89	24	83.69	24	77.72
Error b	162	5736.55	162	812.36	161	460.44

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table I

Analysis of variance for root dry weight at each sampling depth

Source	Depth					
	0.0 - 5.1 cm		5.1 - 10.2 cm		10.2 - 20.3 cm	
	df	Analysis of variance partial sums of squares	df	Analysis of variance partial sums of squares	df	Analysis of variance partial sums of squares
Block	3	61.19*	3	3.83	3	4.61*
Nitrogen	2	65.16**	2	0.92	2	1.34
none vs. medium and high	1	61.71**	1	0.88	1	0.41
medium vs. high	1	3.45	1	0.05	1	0.94
Cutting management	2	26.34	2	0.25	2	1.08
2.5 cm vs. 5.1 and 7.6 cm	1	24.50*	1	0.05	1	0.31
5.1 cm vs. 7.6 cm	1	1.84	1	0.20	1	0.77
Error a	24	108.88	24	11.71	24	9.99
Date/Year	6	193.64***	6	19.29***	6	15.17***
April 15, 1999 vs. May 27, 1999 to October 13, 2000	1	86.25***	1	0.16	1	0.74
1999 vs. 2000	1	36.82**	1	1.06	1	0.03
September 22, 1999 vs. May 27 and October 25, 1999	1	6.81	1	1.68*	1	2.37**
May 27, 1999 vs. October 25, 1999	1	21.76*	1	8.83***	1	11.00***
August 28, 2000 vs. May 30 and October 13, 2000	1	3.67	1	1.40*	1	0.18
May 30, 2000 vs. October 13, 2000	1	38.34**	1	6.00***	1	0.69
Nitrogen x Date	12	50.32	12	5.85	12	2.71
Cutting management x Date	12	83.43	12	4.57	12	3.73
Nitrogen x Cutting management x Date	24	66.15	24	6.61	24	7.24
Error b	162	678.98	161	49.70	160	34.47

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table J**Analysis of variance for root length at each sampling depth**

Source	df	Depth		
		0.0 - 5.1 cm	5.1 - 10.2 cm	10.2 - 20.3 cm
		Analysis of variance partial sums of squares		
Block	1	16718342.87**	3786281.23**	3007331.69**
Nitrogen	1	1318500.71	1700.06	55467.13
Cutting management	1	3328996.37*	98355.23	66954.49
Error a	3	911601.81	325500.43	98183.95
Date/Year	6	30855694.35***	2403566.84**	2416238.65**
April 15, 1999 vs. May 27, 1999 to October 13, 2000	1	974643.02	207598.41	496971.66*
1999 vs. 2000	1	12160040.07**	69582.41	267998.22
September 22, 1999 vs. May 27 and October 25, 1999	1	1263969.71	60927.30	1794.75
May 27, 1999 vs. October 25, 1999	1	2139659.50	299219.94	458122.54*
August 28, 2000 vs. May 30 and October 13, 2000	1	5168526.58**	595466.44**	684517.83**
May 30, 2000 vs. October 13, 2000	1	9148855.46**	1170772.69**	506833.65*
Nitrogen x Date	6	1565227.72	560133.31	879429.64
Cutting management x Date	6	2742384.52	582218.75	975329.69
Nitrogen x Cutting management x Date	6	960105.28	262711.82	109386.56
Error b	24	15245233.72	2118012	2222797.49

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

Appendix Table K

Analysis of variance for soil NO₃⁻ for 1999 and 2000

Source	1999		2000	
	df	Analysis of variance partial sums of squares	df	Analysis of variance partial sums of squares
Block	3	0.09	3	1.10
Nitrogen	2	2.93*	2	10.37*
none vs. medium and high	1	2.13*	1	10.06*
medium vs high	1	0.80	1	0.31
Cutting management	-----	-----	2	11.63*
2.5 cm vs. 5.1 and 7.6 cm	-----	-----	1	11.41**
5.1 cm vs. 7.6 cm	-----	-----	1	0.22
Nitrogen x Cutting management	-----	-----	4	7.81
Error a	6	1.37	24	34.82
Date	1	1.56**	1	26.22***
Nitrogen x Date	2	0.07	2	7.84
Cutting management x Date	-----	-----	2	1.65
Nitrogen x Cutting management x Date	-----	-----	4	20.58**
Error b	9	0.85	27	34.82

Note: Significance of F test. *, P < 0.05; **, P < 0.01; ***P < 0.0001.

Appendix Table L

Analysis of variance of soil pH and available soil P, K, Ca and Mg in experimental plots in 1999 and 2000

		pH	P		K	Ca	Mg
Source	df	Analysis of variance of partial sums of squares		df	Analysis of variance of partial sums of squares		
Block	3	1.26***	2865.69***	3	78009.59***	5697669.62	11931.61***
N	2	0.17	537.50	2	4173.06	2993296.99	1655.92
none vs. medium and high	1	0.02	78.13	1	3512.15	2396915.66	27.21
medium vs. high	1	0.16	459.38	1	610.48	637097.46	1635.34
Cutting Management	2	0.05	237.04	2	973.22	640525.23	457.60
2.5 cm vs. 5.1 and 7.6 cm	1	0.05	236.53	1	61.53	499753.84	432.44
5.1 cm vs. 7.6 cm	1	0.00	0.51	1	907.64	145256.92	26.94
N x Cutting Management	4	0.46	650.21	4	12303.07	4710441.54	2860.74
Error a	24	1.63	3752.25	24	236255.10	36513635.28	23033.18
Year	1	0.24**	1501.56***	1	3317.52	28377034.03***	26696.60***
N x Year	2	0.01	8.17	2	12573.80*	774782.99	484.85
Cutting Management x Year	2	0.03	60.79	2	29831.66**	6134593.71	564.97
N x Cutting Management x Year	4	0.14	161.29	4	2392.59	5234267.41	2590.16
Error b	99	2.20	5072.44	95	165772.26	118709883.52	41531.91

Note: Significance of F test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.0001$.

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