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Effects of Nitrogen Fertilizer and Red Clover (*Trifolium pratense* L.) Component on Productivity of Grass-Legume Mixed Swards

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Thesis submitted to the
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ABSTRACT

Effects of Nitrogen Fertilizer and Red Clover (*Trifolium pratense* L.) Component on Productivity of Grass-Legume Mixed Swards

Chamil Salinda Kandapola

The effects of two levels of red clover (low and high) and three levels of N fertilizer (0, 28, 56 N kg ha^{-1}) on herbage accumulation and quality of grass-legume mixed swards were studied. Total herbage accumulation of the mixtures with high legume percentage was significantly higher than those with low legume percentage and this was most pronounced at second harvest. Swards high in legumes also had higher crude protein concentrations and lower weed percentages than those low in legumes. Although the effect of N on herbage accumulation was not significant, an increase was observed for crude protein concentration of the grass fraction at the higher N levels. A reduction in legume percentage was observed with increasing levels of N. It was concluded that presence of 25 to 35% red clover in the sward increases total herbage accumulation.

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INTRODUCTION

Raising livestock is common among most West Virginia farmers. Natural and naturalized grasslands are a common feature throughout the hilly terrain of the Appalachian landscape. These grasslands are either used for grazing or for hay production. Increasing productivity while conserving the environment in these low-input farming systems is an important priority in developing them sustainably.

Unlike high input monoculture-based farming, most of the pastures in West Virginia contain more than one species of grass. These grasslands also include some legumes such as white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.). These legumes, when grown with grasses as mixed swards, offer several advantages. Compatible grass legume mixtures usually yield more than any single component grown in monoculture (Dobson and Beaty, 1977). Because of their nitrogen fixing ability, the nutritional quality of legumes is often superior to grasses (Weller and Cooper, 2001). It is also believed that the grass can benefit from the N fixed by the legume (Mallarino et al., 1990a; Thomas, 1992). Nitrogen is transferred between the root systems by death and decay of legume roots and nodules and by nitrogenous compounds released from the nodules (Gil and Fick, 2001). The extent of the advantages depends on the species and the amount of legumes present in the mixed sward.

A major problem encountered with legumes is their persistence (Bryan, 1985; Brummer and Moore, 2000). Maintaining an adequate legume stand for a long period of time in a mixed sward is a difficult management task. Because legumes lack persistence, grasses grown with legumes are unable to benefit from legume N for more than a few years.

Fertilizer N use is a common and easy alternative to legumes. Nitrogen is the most limiting nutrient in world crop production. Appling N to West Virginia pastures is not common; however, it is commonly applied to hayfields. It is known that excessive application of fertilizer N will lead to suppression of legume N fixation (Tanner and Anderson, 1964). Grasses and legumes respond differently to frequency and rate of applied nutrients. Growth of legumes such as clover, is depressed by high N levels in soil through competition from the accompanying grasses (Belesky et al., 2000), resulting in less symbiotic N fixation. Nitrogen fertilizer management plays an important role in maintaining an adequate stand of legume and grass. Thus, it is important to minimize the use of N fertilizer in grass-legume pastures. In intensive harvesting and grazing systems a persistent stand of legumes with grasses is an important aspect of maintaining productivity and quality of the grassland.

Legumes and N, and their effects on quality and quantity of the forage have been thoroughly investigated during the past few decades throughout the world (Panditharatne et al., 1986; Boller and Nosberger, 1987; Sleugh et al., 2000). Most of this research was focused on seeded binary grass-legume mixtures. Much is known about the effect of high levels of N on these, less diverse mixtures. Few studies have provided data on the effects of red clover and low levels of N on low input permanent grassland with several different species. Therefore, this study focused on the effects of legume percentage and low levels of fertilizer N on West Virginia grassland.

The objective of this study was to determine the effects of different percentages of red clover and low levels of N fertilizer on herbage accumulation, botanical composition, and crude protein concentration of grass-legume swards.

REVIEW OF LITERATURE

Role of Grass-Legume Mixtures in Livestock Production

Growing legumes with grasses in mixed swards has been practiced over the centuries throughout the temperate world (Haynes, 1980). It is commonly known that high yields can be obtained by including legumes with grasses as mixed swards (Dobson et al., 1976; Olsen et al., 1981; Barnett and Posler, 1983; Scheneiter, 1999). In addition, there is a good possibility of providing higher quality forage than grass alone, which is important in animal nutrition. Grass-legume mixtures yield more dry matter, more protein, and yield is better distributed compared to grass grown alone (Brown and Munsell, 1943; Dobson and Beaty, 1980; Barnett and Posler, 1983; Weller and Cooper, 2001). Field stockpiling can be done with grass-clover mixtures to provide animals with good quality forage towards the end of the season (Collins and Taylor, 1984; Sheaffer et al., 1984), which will also reduce hay harvesting and storage costs.

Legumes such as red clover are often used in mixtures with entophyte infested tall fescue to dilute the toxic effects of the endophyte (Lomas et al., 1999). Weed invasion in grass or legume stands can be controlled by converting them into grass legume mixtures, which can reduce the use of herbicides (Sheaffer et al., 1984). The renewed interest in legumes as a substitute to fertilizer N has resulted in lowering the N inputs in grassland farming. In addition, harvesting biologically fixed N as mixed swards will lower the need for concentrate feed in livestock farming. These economic and environmental advantages of mixed swards make them a viable component in a sustainable agricultural system.

Role of Legumes in Mixed Swards

In low-input grassland farming, lowering the use of N fertilizer is a high priority. Nitrogen economy is the main advantage of having legumes in a mixed sward. For example, Weller and Cooper (2001) showed that white clover-perennial ryegrass mixtures can produce a yield equal to ryegrass alone with 336 kg N ha^{-1}. Legumes exert little competition with grasses for soil N when they are actively fixing biological N (Nannipieri et al., 1985). In addition, grass can benefit from biologically fixed N released from legume roots into the soil (Laidlaw et al., 1996).

Legumes can be successfully used in grassland renovation. The productivity and quality of the grasslands can be improved by sod-seeding legumes into the existing grass stands (Bryan, 1985; Olsen et al., 1981).

Legumes are important in maintaining productivity and quality of the soil. Inclusion of legumes has a positive effect on the amount and rate of N mineralization in soil (Gill and Fick, 2001). Because of their higher litter quality, legumes can be successfully used in soil improvement. Most of the biologically fixed N is liberated after death and decay of the root nodules (Dilz and Mulder, 1962). Under grazing conditions, biologically fixed N in legumes becomes available in the soil via excreta of animals.

Legume proportion is an important factor determining the amount of N transfer to grass-forage of a mixture, and the benefits associated with it. For pure legume stands, percentage N derived from biologically fixed N has a negative correlation with herbage yield (Boller and Nosberger, 1987). Per plant N fixation by legumes grown with grasses is greater than that of legumes grown alone due to uptake of available soil N by accompanying grasses, and lower intra-specific competition for available resources

(Montes et al., 1983). Panditharatne et al. (1986) showed that there is no effect on nutritional composition of the grass forage when the percentage of legume in the mixture is low (8%). However, N transfer is more efficient in mixtures with lower legume stands due to the efficient N utilization by the abundant grasses (Mallarino et al., 1990a). In general, a 30% legume stand is considered as the threshold between high and low when determining optimum legume percentage in a mixed sward (Miller and Reetz, 1995).

Red Clover

Red clover is an herbaceous short-lived perennial and belongs to the family Fabaceae, sub-family Papilionoideae. It is a high yielding forage legume that is usually grown in mixed swards with grasses. Red clover is used as pasture, hay or silage and is an important green manure in crop rotations. It is grown in a wide range of climatic and soil conditions throughout the temperate regions of the world. Red clover stands can persist about four years under favorable conditions. Red clover is easy to establish relative to most pasture legumes because of its high seedling vigor.

In red clover, percentage N derived from symbiosis averages between 61 and 96% of total plant N (Boller and Nosberger, 1987; Warembourg et al., 1997). Red clover can yield over 200 kg N ha^{-1} yr^{-1} without N fertilizer in a well-managed mixed sward, having more than 50% clover stand. Under such conditions, N transfer from clover to grass has been estimated to be about 40 kg ha^{-1} yr^{-1} (Boller and Nosberger, 1987). The amount of N transfer depends on the percentage of legume and grass in the sward (Boller and Nosberger, 1987; Mallarino, et al., 1990b). It has been estimated that 2.41 to 4.59 kg ha^{-1} yr^{-1}of fixed N is transferred to grasses per unit percentage red clover in the mixture.

However, a higher amount of N in grasses with increasing clover content can also be due to decreasing grass percentage in the mixture. Boller and Nosberger (1987) and Warembourg et al. (1997) also showed that N fixation in red clover is more consistent throughout the season compared to other legumes such as white clover. However, Warembourg et al. (1997) observed a seasonal pattern of N fixation in an uncut red clover stand. Nitrogen fixation had two peaks, in spring and late summer, and a sharp drop in biological N fixation at flowering.

Mallarino, et al. (1990a) observed a linear increase in proportion of grass N derived from legume, with increasing legume proportion in a red clover-tall fescue mixture. In the same study, N transfer was greater in the second year than in the seedling year and averaged 37% and 24% of total grass N respectively. The amount of N transferred to grass did not increase with increasing legume proportion due to the reduction in the grass component. The study indicates that utilization of symbiotically fixed N can be limited because of low grass presence in the swards with higher legume stands.

Regardless of its superiority, use of red clover has been restricted mainly due to the higher management skills required, lack of persistence, tendency to cause bloat in cattle and level of estrogenic compounds (Bowley et al., 1984), which affect the fertility of ewes.

Persistence of Red Clover in Mixed Swards

Although red clover is a high yielding forage, the main drawback associated with it is its lack of persistence. Maintaining a proper balance of legumes for a long period of time is a major challenge in mixed sward production. For example, persistence of red clover is limited to three years under most of the pasture management systems in the northeastern United States (Prigge et al., 1999). Although red clover produces high yields in the first year of production, stand decrease was observed in subsequent years (Dobson et al., 1976; Olsen et al., 1981). Similar observations of Bryan et al. (1985) indicate that the persistence of red clover in grass swards starts to decline in the second year after establishment. Competition from accompanying grasses, unfavorable climatic conditions, insects, nematodes and diseases such as anthracnose, powdery mildew, crown rot, and root rot are the major causes of low persistence (Skipp and Christensen, 1990). In addition, landscape position, defoliation, and fertilizer management also affect persistence (Harmoney et al., 2001; Fribourg et at., 1984).

Harris et al. (1980) reported a dominance of red clover in mixed swards in the second year after establishment. A similar observation was reported by Olsen et al. (1981) where red clover suppressed grass growth during the first production year. An alternative pattern of grass and clover dominance was observed by Loiseau et al. (2001) in ryegrass white clover swards. This pattern was attributed to the N supplying capacity of the soil. Depletion of soil N levels, as a result of high grass growth in the first year, favors legume growth in the following year. This will result in clover dominance. The higher clover content in the second year will increase soil N status, resulting in higher grass growth and lower legume growth in the following year.

Improper fertilizer management can affect legume persistence. Application of N fertilizers to mixed swards is a major factor affecting persistence (Kresge, 1964; Stout et al., 2001). Proper N fertilizer and defoliation management schemes are important in maintaining a favorable stand composition.

Defoliation Management in Mixed Swards

Maintaining an optimum leaf area index in order to obtain maximum yield of best quality is the primary objective of defoliation management. Frequency and intensity of defoliation affect productivity and persistence of the sward. In mixed swards, it is important to maintain a proper sward height to reduce competition for light from grasses on legumes. Defoliation is done either by cutting or by grazing. Grazing can be continuous or rotational. Continuous grazing at short (< 5 cm) heights can have a severe impact on stands of red clover (Brummer and Moore, 2000). Rotational grazing management proved to extend the life of legumes (Smith et al., 1985). However, Harmoney et al. (2001) showed that both continuous and rotational grazing will increase the legume component in a mixed sward. They found that the persistence of red clover was better under rotational grazing while that of white clover was better under continuous grazing. The increase in legume component was a result of reduced competition from grasses due to the disturbance of the sward canopy by grazing. Stout et al. (2001) suggested that maintaining a 15 cm sward height would maximize the clover fraction in white clover-orchardgrass mixed swards. However, Dobson et al. (1976) showed that there was no significant difference in yield or persistence of red clover in tall fescue mixed swards between clipping heights of 5 and 10 cm. Harris et al. (1980) recorded a 40% lower production under frequent grazing of red clover-ryegrass mixtures compared to infrequent grazing. However, they did not observe a difference in percentage of red clover in response to grazing frequency.

Red clover, with an upright growth habit, is less tolerant of close and frequent grazing because it has more growing points and leaves in the upper canopy. Close grazing can result in a loss of a higher proportion of growing points and leaf area. Most of the C requirement of the roots is supplied by the rosette leaves at the base of the red clover plant (Warembourg et al., 1997). Translocation of assimilates to red clover roots was estimated as one third of the total assimilates of the plant in the vegetative phase (Fernandez and Warembourg, 1997). More red clover can be harvested by growing it with tall grasses such as orchardgrass than short pastures such as Kentucky bluegrass. Prigge et al. (1999) observed that early spring grazing followed by late fall grazing lowered the production of red clover. However, they observed an increase in red clover percentage in fall grazed hay fields compared to fall harvested hay fields.

Nitrogen Application to Mixed Swards

Mineral N is known to reduce the proportion of legumes in a mixed sward (Robinson and Sprague, 1946; Kresge, 1964; Woledge, 1988). Lack of persistence of legumes when grown with N fertilized grass may be caused by competition between grass and legume due to the increasing grass to legume ratio in the mixture. Grasses grow more vigorously than legumes in the presence of high soil N levels, eventually shading the legumes. The negative effect of N fertilizer application on N fixation by legumes is highly correlated with the amount of N applied (McAuliffe et al., 1958). Supplemental N also has a negative effect on N transfer from clover to grass. Boller and Nosberger (1987) observed a 33% decrease in clover N transferred to grass after applying 120 kg N ha^{-1} yr^{-1}. They also showed that the amount of N transferred was affected more severely than N fixation by the legume. Dennis and Woledge (1985) observed a reduction in the leaf area of white clover due to fewer stolons when ryegrass-white clover mixtures were supplied with N fertilizer. Ryle et al. (1981) showed that the lack of persistence of legumes grown in mixed swards fertilized with high N levels was related to root to shoot ratio of the legume. They observed a decrease in root to shoot ratios when nodulated legumes were supplied with nitrate N. High N rates increase soil acidity (Haby et al., 1999). This can indirectly affect the persistence of legumes because legume growth and persistence is negatively affected by soil acidity (Barnard and Folscher, 1988).

The amount of N fixed by legumes is usually lower than the amount that grasses need for maximum production, especially during the early spring when grass growth is rapid. Early season N application is a major focus in strategic use of N fertilizers without reducing biological N fixation (Stout et al., 2001; Frame and Boyd, 1987). Early season N application before the onset of favorable temperatures for N fixation can minimize negative effects on the legume while providing much needed N for early growth of cool season grasses. Stout et al. (2001) observed a 50% decrease in the white clover fraction at 89.6 kg N ha^{-1} after applying N in early spring. However, they observed a 20% yield increase at a N rate of 45 kg ha^{-1}, although the clover fraction decreased compared to the lower N treatment.

Kresge (1964) showed that it is not economical to apply N fertilizer to red clover-orchardgrass mixtures with more than 20% clover stand. According to his findings, clover content decreased while there was no yield increase at N rates ranging from 0 to 100 lb ac^{-1}. However, he found that if the clover percentage was less than 20, the grass-legume mixture behaved like a pure grass sward and N fertilizer could be applied to increase yield with no effect on the clover stand. In the same study, total herbage N concentration was not affected by added N or by the legume percentage. Similar observations were made by West et al. (1980). A negative relationship between grass percentage of the mixture and the crude protein concentration of the grass component was reported by Barnett and Posler (1983). However West et al. (1980) found that moderate levels (30 to 60 kg ha^{-1}) of N fertilizer were beneficial in the establishment year when the legume was interseeded into permanent pastures on N deficient soils. They observed that seedling vigor, legume yield at first cut, and total yield were higher under moderate N levels and lower at N rates above 60 kg ha^{-1}. Benefits of low levels of N on grass-legume mixtures were also documented by Stout et al. (2001) and Kunelius (1974).

Effects of Combined Nitrogen on Nitrogen Fixation in Legumes

It is widely known that addition of high levels of combined N (NO_3^- and NH_4^+) to the root medium can inhibit nodulation and N fixation in legumes. Effects of combined N depend on host species, variety, growth stage, form (NO_3^- or NH_4^+) and amount of nitrogen supplied (Harper and Gibson, 1984; Latimore et al., 1977; Tanner and Anderson, 1964). For example, subterranean clover (*Trifolium subterraneum* L.) is less sensitive to the inhibitory effects of nitrate than soybean (*Glycine max* L).

Both NO_3^- and NH_4^+ have inhibitory effects on N fixation (Tanner and Anderson, 1964). Nitrate-N generally shows more negative effects than ammonium N (Streeter, 1982). Nitrate is the form of N absorbed in greatest amounts by plants from soil. Tanner and Anderson (1964) found that locally placed NO_3^- has a direct inhibitory effect on nodulation at the site of placement. They have shown that nitrate supplied to one part of the root system had no effect on infection in the other part, although the nitrate rich part supplied adequate nitrogen nutrition to the plant. In the presence of combined N, reduction of root hair curling and the formation of fewer infection threads have been observed. Indole acetic acid (IAA) is important in root hair infection. Tanner and Anderson (1964) showed that in the presence of NO_3^-, IAA can be destroyed by the NO_2^- produced in nitrate reduction. The rate of production or destruction of IAA ultimately determined the time taken for infection and nodule formation.

Day et al. (1989) showed that the inhibitory effect of NO_3^- on nodule initiation and development in soybean depends on an interaction between NO_3^- and the autoregulation signals produced by the shoot. A grafting experiment with two soybean mutants indicated that they have an altered auto-regulatory signal that originated in the shoot in response to the amount of added NO_3^- in the root medium.

Some products of nitrate reduction are believed to be inhibitory to nitrogenase activity in root nodules. Inhibition of nitrogenase activity by the accumulation of NO_2^- is a good possibility. Addition of NO_3^- rapidly induced the senescence of root nodules in alfalfa (*Medicago sativa* L.) by the degradation of bacterial proteins (Becana et al., 1985). Streeter (1982) observed different levels of accumulation of NO_3^- and NO_2^- in the nodules of soybean plants fed with different levels of NO_3^-. His results showed that a high NO_3^- supply in nutrient solution (100 mg l^{-1}) significantly altered the growth rate of nodules in sand culture. Accumulation of high concentrations of NO_3^- and low concentrations of NO_2^- in nodules accompanied the depression of nodule growth by NO_3^-.

Reduction of atmospheric N_2 by nitrogenase and NO_3^- reduction by nitrate reductase have fairly similar carbohydrate requirements. For example, 5.9 mg C is used to fix 1 mg of N and 6.2 mg C is used to reduce 1 mg of N in the process of N assimilation (Minchin and Pate, 1973). Because of the high C cost of N fixation, in most cases, NO_3^- uptake can dominate N fixation (Sawhney and Singh, 1985). To a lesser extent, N fixation can suppress NO_3^- uptake. This was illustrated by Henning (1995) in NO_3^- fed faba bean (*Vicia faba* L.) plants. Inhibition of nodule development and nitrogen fixation by applied N is also caused by an insufficiency of photosynthate received by the nodules. This was attributed to use of carbohydrates in nitrate assimilation and allocation of carbohydrates to top growth following ready availability of N. This has been shown by reduced translocation of ^{14}C labeled photosynthetic products from leaves to nodules of NO_3^- fed plants (Latimore et al., 1977). Nitrate fed legumes also exhibited low nitrogenase activity in the same study.

Arrese-Igor et al. (1997) observed a 23% decrease in N fixation in nodulated soybean plants in the presence of 0.01 *M* NO_3^- solution compared to the nitrate free control. This accompanied a reduction of nodule mass. They also observed a reduction in nitrogenase-linked respiration and a steady increase in C cost of nitrogen fixation in the presence of NO_3^-. The presence of NO_3^- also caused nodule oxygen diffusion resistance to increase, reducing availability of oxygen for respiration of *Rhizobium*, leading to a reduction in N fixation.

Positive effects of low levels of NO_3^- on nodulation and early growth of legumes had been reported by Butler and Ladd (1985). Waterer et al. (1992) observed that at low concentrations (< 1.0 m*M*), NH_4^+ increased N fixation in field peas (*Pisum sativum* L.).

The role of NO_3^- metabolism in biological N fixation is highly controversial. There is no accepted single reason for the inhibitory effect of N. A combination of several factors can lead to the overall inhibition of nodulation and fixation of nitrogen in legumes. However, it can be concluded that added nitrogen can reduce, have no effect on, or occasionally increase nitrogen fixation by legumes depending on the species, amount of soil N, and the growth stage of the legume.

MATERIALS AND METHODS

Experimental Sites

The experiment was conducted in 2000 and 2001 in northern West Virginia. The experimental areas were located on the gently sloping (5 to 10 % slope) areas of hilly grasslands in the Appalachian Mountains. There were three locations. One at Morgantown was in a permanent grassland pastured the previous year. Two locations were in a permanent hayfield at Reedsville. These grasslands were composed of the following grass species, orchardgrass (*Dactylis glomerata* L.), tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire), Kentucky bluegrass (*Poa pratensis* L.), and timothy (*Phleum pratense* L.). Red clover (*Trifolium pratense* L.) was the prominent legume in the experimental areas, while white clover (*Trifolium repens* L.) was also present in relatively small amounts. Broadleaf weedy species, primarily bedstraw (*Galium aparine* L.), plantain (*Plantago* spp.) and dandelion (*Taraxacum officinale* L.) were also present in substantial amounts in the experimental areas and throughout the grasslands.

Location I (Morgantown 2000)

Location 1 (Appendix Figure A) was situated on the West Virginia University Livestock Farm, Morgantown, WV ($39^{o}.39^{/}$ N, $79^{o}.55^{/}$ W; 370 m above sea level). The experimental plots were situated on the Clarksburg soil series (fine-loamy, mixed, mesic Typic Fragiudalfs).

Locations 2 and 3 (Reedsville 2000 and 2001)

Locations 2 and 3 (Appendix Figures B and C) were situated on the West Virginia University Experimental Station, Reedsville, WV ($39^{o}.51^{/}$ N, $79^{o}.81^{/}$ W; 550 m above sea level). The experimental plots were situated on the Gilpin soil series (fine-loamy, mixed, semiactive, mesic Typic Hapludults).

Experimental Design

The experiment was conducted as a two-factor factorial in a split-plot design. Experimental sites were chosen based on the botanical composition of the existing sward, mainly the legume component. Legume density was the main plot treatment and N fertilization was the sub-plot treatment. There were two main plot and three subplot treatments per block. Main plot treatments were low (0 - 5% red clover in the sward) and high (30 - 50 % red clover in the sward) legume stands. Sub-plot treatments were three N levels, 0, 28 in a single application and 56 kg N ha^{-1} applied in two equal split applications. Nitrogen was manually broadcasted onto the plots in the form of granular urea. Nitrogen application dates are summarized in Table 1. Soil samples were taken from 0 to 5 cm and K and P fertilizer applications made according to soil test results (Appendix Table A). Experimental procedures were the same at all three locations except that the number of blocks were four, eight and six in location 1, 2, and 3, respectively.

Table 1. Nitrogen fertilizer application dates for each location.

	Location		
N application	Morgantown 2000	Reedsville 2000	Reedsville 2001
	Date		
Application 1	April 19	April 20	April 25
Application 2	August 25	August 23	August 17
Application 1	April 23 (2001)	April 25 (2001)	April 30 (2002)

Field Layout

The same field layout was used at all locations in each year. Two main plots (4 x 6 m) with desired legume densities were allocated to each block. Three levels of N were allocated randomly to the sub-plots (2 x 4 m) within each main plot. Figure 1 is a schematic representation of a main plot. Main plots at each location were located less than 30 m from each other to minimize experimental error (Appendix Figures A, B and C). At location 1 main plot 2 was on an area reseeded with red clover in 1998. Main plot 1 was on a permanent grassland area.

Data Collection

1. Herbage Accumulation

Three harvests were made at each location each year (Table 2). First cut was made in late May or early June in order to obtain maximum yield from the spring growth. A second cut was made in mid summer (late July) to harvest the early summer growth and a third cut was made in mid October to obtain late season growth. A spring harvest was made at each location in the following year to determine treatment effects on botanical composition and herbage accumulation into a second year.

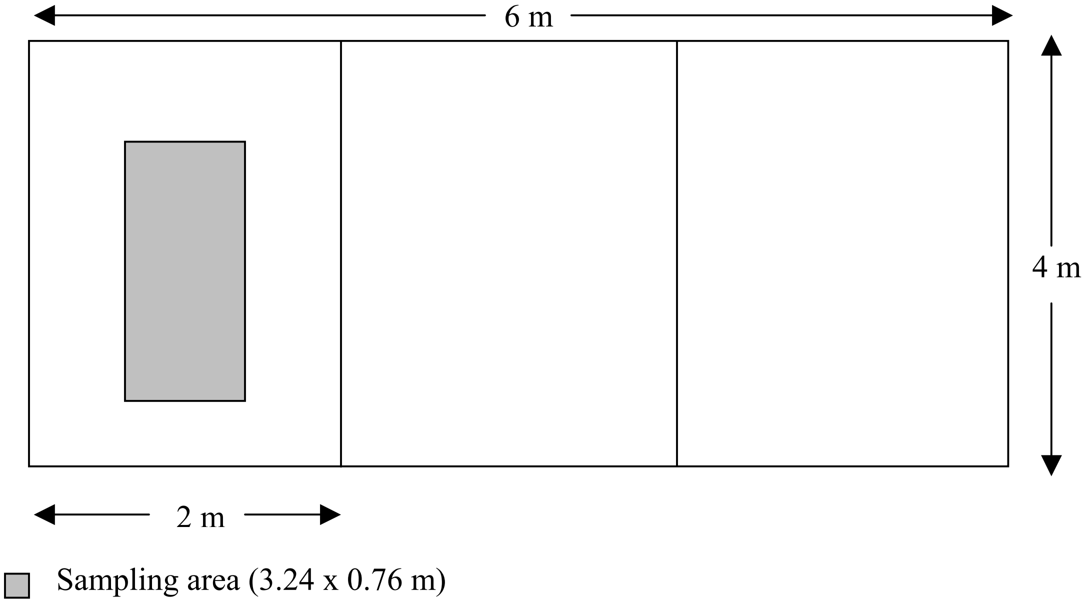

Figure 1. Diagram of a main plot showing sub-plots and sampling area.

The ends of each sub-plot were mowed prior to sampling to obtain a uniform sampling area. Then, a 3.24 m strip along the center of each sub-plot was cut at 5 cm using a sickle bar mower (0.76 m blade width). Cut herbage was weighed in the field, and a sub sample was taken for determination of botanical composition, moisture, and N concentration. Herbage accumulation was calculated as follows,

$$\text{Herbage accumulation (kg ha}^{-1}\text{)} = \frac{\text{Field sample wet weight (kg)}}{\text{Sampling area (2.46 m}^{2}\text{)}} \text{ x 10 000 x Dry matter \%}$$

After sampling, the experimental area was mowed and cut herbage was removed.

Table 2. Harvest dates.

	Location		
Harvest	Morgantown 2000	Reedsville 2000	Reedsville 2001
	Date		
Harvest 1	May 25	June 14	June 13
Harvest 2	July 13	July 26	August 7
Harvest 3	October 19	October 11	October 18
Harvest 4	June 21 (2001)	June 17 (2001)	June 20 (2002)

2. Botanical Composition

Botanical composition was estimated visually immediately before harvest. After harvesting, the sample of cut herbage was hand separated into grass, legume, weeds and dead material. Each component was oven dried for 48 hours at 65° C and weighed. The composition of each component was calculated as g kg^{-1} of dry matter.

3. Crude Protein

The grass and legume fractions of harvested herbage from two replicates were separately analyzed for total N (Horneck and Miller, 1998). Dried samples were ground in a Wiley mill to pass a 1 mm screen and 0.05g was used for N analysis. Nitrogen was analyzed by using a Tecator Kjeltech 1030 auto analyzer. Crude protein concentration was calculated by multiplying total N by 6.25, expressed as g kg^{-1} of dry matter.

4. Soil Sampling

At the end of the each growing season, soil was sampled to 5 cm depth. A total of five samples were taken along the center line of each sub-plot by using a tube. Soil samples were analyzed for pH and available K, P, Ca, and Mg (Ghazi et al., 1978). Results of the soil analysis were presented in Appendix Table B.

5. Weather Data

Precipitation (Table 3) and temperature (Table 4) for the 2000 and 2001 (April to October) growing seasons were obtained from the Morgantown Municipal Airport which is located about 1 km south of location 1 and West Virginia University Experimental Station at Reedsville, West Virginia, located about 1 km west of locations 2 and 3.

6. Statistical Analysis

Statistical analyses were performed separately for each location. Data were analyzed by using the general linear models procedure of SAS statistical software (SAS, 1990). Effects of legume stand and N on herbage accumulation, botanical composition, and crude protein concentration were tested. Main plot effects were tested against error a (block x legume) while sub-plot effects were tested against error b (block x legume x N). Single degree of freedom comparisons were performed for N vs. no-N treatments and 28 kg N ha^{-1} vs. 56 kg N ha^{-1} when F tests were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Weather

Precipitation and temperatures from April 1 to October 31 for the three locations are summarized in Tables 3 and 4. The temperature patterns at Reedsville were consistent in 2000 and 2001. Temperatures at Morgantown were somewhat higher compared to Reedsville, mainly because of the difference in elevation. However, favorable temperatures prevailed throughout the growing seasons during the experiment. Rainfall patterns were consistent and evenly distributed over the two years in Reedsville. However, Morgantown experienced a lower amount of precipitation from July through September.

Table 3. Monthly precipitation during the growing season at experimental sites.

Month	Location				
	Morgantown 2000	Morgantown Normal[1]	Reedsville 2000	Reedsville 2001	Reedsville Normal[1]
	mm				
Apr.	116	94	164	100	90
May	120	100	113	129	88
June	109	102	187	127	83
July	91	107	213	187	87
Aug.	80	101	100	88	83
Sept.	64	90	122	112	80
Oct.	41	71	50	49	132
Total	621	665	949	792	643

[1] 30-year averages.

Table 4. Monthly average ambient temperature during the growing season at experimental sites.

Month	Location Morgantown 2000	 Morgantown Normal[1]	 Reedsville 2000	 Reedsville 2001	 Reedsville Normal[1]
	———	———	C °	———	———
Apr.	10.7	10.8	8.1	10.6	10.3
May	17.8	16.3	15.1	14.0	14.1
June	21.5	20.6	19.3	18.1	18.7
July	21.1	22.7	18.9	18.9	20.9
Aug.	20.8	22.0	19.0	20.7	20.1
Sept.	17.4	18.6	15.6	14.6	16.4
Oct.	13.1	12.3	10.7	9.3	10.5
Average	17.5	17.6	15.2	15.2	15.9

[1] 30-year averages.

Herbage Accumulation

A significant increase in total herbage accumulation was observed at all three locations for the high compared to the low legume treatment. By the end of the growing season, the high legume treatment yielded 68, 10 and 18% more dry matter than the low legume treatment at location 1, 2 and 3, respectively (Tables 5 and 6). There was no change in herbage accumulation due to N fertilization. The N x legume interaction was not significant at any of the locations (Table 5 and Appendix Table C, D and E). Results from all three locations indicate that the highest yield obtained in the low legume treatment with 56 kg N ha^{-1} was still lower than the yield in high legume mixtures without N fertilizer.

Table 5. Significance of *F* tests for legume, N and harvest.

	Location		
	Morgantown 2000	Reedsville 2000	Reedsville 2001
Legume	**	*	**
N	NS	NS	NS
Legume x N	NS	NS	NS
Harvest	***	***	***
Harvest x Legume	***	NS	***

*, **, *** : *F*-tests were significant at, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.
NS : Non significant

Treatment effects on herbage accumulation for each harvest at locations 1, 2 and 3 are summarized in Tables 7, 8 and 9. Effect of harvest was significant at all three locations. There was a significant harvest x legume interaction (Appendix Tables F, G and H). However, harvest x N and harvest x legume x N interactions were not significant at any of the locations. The first harvest contributed more than 50% of the total full-season yield at all three locations. The effect of legume on herbage accumulation was most prominent in harvest 2 at location 1 (Table 7). At location 1, the yield increase in high legume plots was 42, 164 and 42% over the low legume stand in harvest 1, 2 and 3, respectively. This can be attributed to the increase in legume component in the second harvest. A somewhat similar effect of legume was observed for location 2 and 3. However, the corresponding increases were 6, 31 and 0 % for location 2 and 27, 32 and 53% for location 3 in harvest 1, 2 and 3, respectively (Tables 8 and 9). The greater increase in herbage accumulation at second harvest at location 1 compared to locations 2 and 3 can be explained by the difference in precipitation in June and July. The total precipitation in June and July was 200, 400, and 314mm at location 1, 2, and 3, respectively. Since red clover is more drought tolerant than grasses it produced more when precipitation was lower.

Table 6. Total herbage accumulation as influenced by levels of legume and N fertilizer.

N rate, kg ha^{-1}	Location					
	Morgantown 2000		Reedsville 2000		Reedsville 2001	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	kg ha^{-1}					
0	7 533	12 524	9 272	10 668	6 327	9 609
28	7 266	12 520	9 692	10 128	8 047	9 287
56 (Split application)	7 709	12 831	9 796	10 754	7 367	9 733
Legume mean	7 503	12 625	9 586	10 517	7 247	9 543
Location mean	10 064		10 052		8 395	

The effect of N fertilization rate on herbage accumulation was not significant at any location nor did it show any trend (Tables 5, 7, 8, and 9, Appendix tables C, D, and E). It can be suggested that the rates of N used were not sufficient to induce any significant yield effect. Timing of N could have been another possibility. Available N levels in the soil are high at the end of the spring due to mineralization induced by increasing temperatures. Application of a small amount of N at the end of April may not have significantly increased available soil N. Splitting the application of 56 kg N ha^{-1} may

Table 7. Effect of N rate, legume content, and harvest on herbage accumulation at location 1.

N rate, kg ha^{-1}	Harvest					
	1		2		3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	kg ha^{-1}					
0	4 576	6 382	1 537	4 097	1 420	2 044
28	4 316	6 509	1 647	4 011	1 302	2 000
56 (Split application)	4 592	6 362	1 505	4 311	1 611	2 157
Legume mean	4 497	6 418	1 563	4 140	1 444	2 063
Harvest mean	5 456		2 851		1 756	

Effect of legume was significant at *P*<0.01.
Effect of harvest was significant at *P*<0.001.

Table 8. Effect of N rate, legume content, and harvest on herbage accumulation at location 2.

N rate, kg ha^{-1}	Harvest					
	1		2		3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	kg ha^{-1}					
0	4 676	5 428	2 041	2 563	2 555	2 676
28	5 249	5 103	1 888	2 534	2 554	2 490
56 (Split application)	4 801	5 154	1 917	2 588	3 077	3 011
Legume mean	4 908	5 229	1 948	2 561	2 729	2 726
Harvest mean	5 069		2 255		2 727	

Effect of legume was significant at *P*<0.05.
Effect of harvest was significant at *P*<0.001.

Table 9. Effect of N rate, legume content, and harvest on herbage accumulation at location 3.

N rate, kg ha^{-1}	Harvest 1		Harvest 2		Harvest 3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	kg ha^{-1}					
0	4 095	6 620	1301	1 662	929	1 327
28	5 845	6 376	1 153	1 362	1 049	1 549
56 (Split application)	5 214	6 315	1 165	1 752	986	1 666
Legume mean	5 051	6 437	1 206	1 592	988	1 514
Harvest mean	5 744		1 399		1 251	

Effect of legume was significant at $P<0.01$.
Effect of harvest was significant at $P< 0.01$.

have also resulted in rates too low to have a significant effect on available soil N. However, there were effects of N on botanical composition and quality of the herbage.

Herbage accumulation at finishing harvest (harvest 4) at three locations is summarized in Table 10. Legume percentages had changed at the finishing harvest so that a comparison of low and high legume was not meaningful. There was no significant effect of legume. However, a significant effect of N was observed at location 2. Herbage accumulation increased with increasing rates of N at location 2. This effect of N was different from the first year at the same location.

Table 10. Herbage accumulation at harvest 4 as influenced by levels of legume and N fertilizer.

N rate, kg ha^{-1}	Location					
	Morgantown 2000		Reedsville 2000		Reedsville 2001	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	kg ha^{-1}					
0	4 983	5 855	5 492	5 294	6 240	6 137
28	5 103	5 656	5 810	6 950	6 561	6 769
56 (Split application)	5 620	6 401	6 252	6 237	6 460	7 138
Legume mean	5 235	5 971	5 852	6 160	6 421	6 681
Significance*						
Legume	NS		NS		NS	
N	NS		**		NS	
Legume x N	NS		*		NS	
Location mean	5 603		6 006		6 551	

*, **, *** : *F*-tests were significant at, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.
NS : Non significant.
Note: Finishing harvests were made in spring 2001 at location 1 and 2, and spring 2002 at location 3.

Botanical Composition

The effect of N on botanical composition was significant only at location 2 (Table 11, Figures 2, 3, and 4, and Appendix Tables I, J and K). At location 2, the legume percentage decreased in treatments with N compared to those without N. A general decrease in legume percentage was also observed with increasing N rates in the high legume stand at location 1 and in the low legume stand at location 2. This can be attributed to the higher response of grasses to applied N compared to legumes and lower response of legumes due to competition from grasses. Although data are not available on soil N status for different sites, it can be speculated that available soil N was higher at location 2 compared to the other locations.

Table 11. Effects of legume, N, and harvest on botanical composition.

	Location											
	Morgantown 2000				Reedsville 2000				Reedsville 2001			
	Grass	Legume	Weed	Dead Material	Grass	Legume	Weed	Dead Material	Grass	Legume	Weed	Dead Material
Legume	NS	***	*	NS	*	***	***	*	NS	***	*	**
N	NS	NS	NS	NS	**	*	NS	NS	NS	NS	NS	NS
Legume x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** : *F*-tests were significant at, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.
NS : Non significant
Note: Legume effect was not independent.

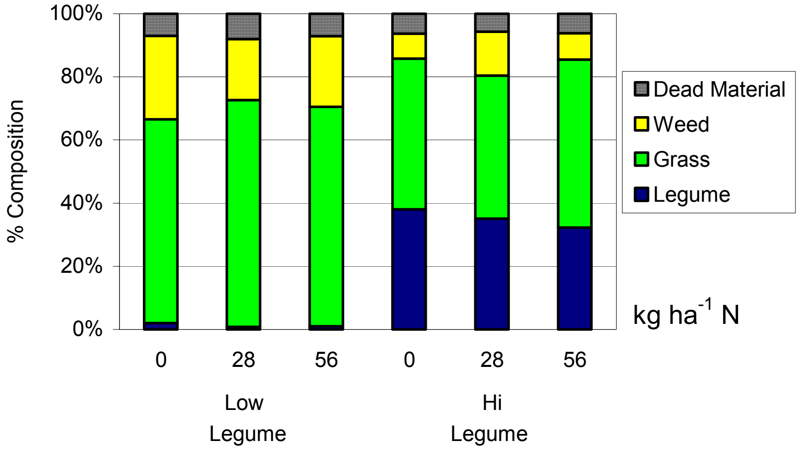

Figure 2. Botanical composition as affected by legume and N at location 1. Effect of legume on weed was significant at *P*<0.05.

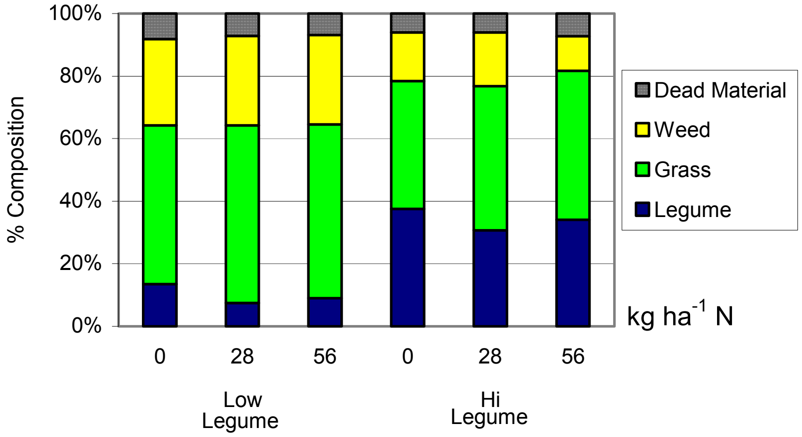

Figure 3. Botanical composition as affected by legume and N at location 2. Note: Effect of legume on grass, weed and dead material was significant at *P*<0.05, *P*<0.001, and *P*<0.05 respectively. Effect of N on grass and legume was significant at *P*<0.01 and *P*<0.05 respectively.

It was also observed that the weed component was significantly reduced by high legume stands at all three locations (Table 11, Figures 2, 3, 4, and Appendix Tables I, J, and K). This can be explained by the higher competition of legumes with broadleaf weeds compared to grasses.

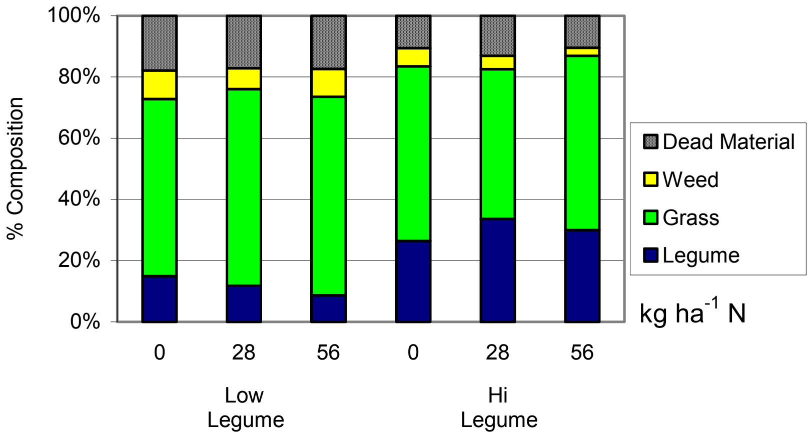

Figure 4. Botanical composition as affected by legume and N at location 3. Effect of legume on weed was significant at *P*<0.05. Effect of legume on dead material was significant at *P*<0.01.

A significant reduction in dead material was observed at locations 2 and 3 in high compared to low legume stands. Presence of less senescenced material with higher percentage clover may be an indication of more stand longevity. However, there was no significant reduction in percentage grass at the higher legume level although the grass fraction was generally higher in low legume stands. The data show that less legume in the stand was compensated by weed and dead material rather than by grass. This shows the importance of maintaining an adequate legume stand in order to control weeds.

Grass and legume percentages in the mixed sward varied significantly over harvests. Figures 5, 6, and 7 show the percentage grass and legume in low and high legume stands at each harvest for each of the three locations. The behavior of the grass and legume fractions through the season showed a very similar pattern at all locations. Harvest 2 showed a marked increase in legume component in both high and low legume stands. This increase was much more prominent in high legume stands, and was mainly the result of reduced competition from grasses on legumes through canopy removal at first harvest. However, at third harvest, a reduction in legume fraction and an increase in grass fraction was observed compared to second harvest. This may have been caused by the cooler and drier conditions that prevailed in September and October, which did not favor legumes. Renewed grass growth towards the end of the season due to favorable temperatures for cool season grasses may have contributed to the reduction in the legume stand. In addition, a killing frost on October 8 and 9 at Reedsville in 2000 and 2001, had a detrimental effect on legumes.

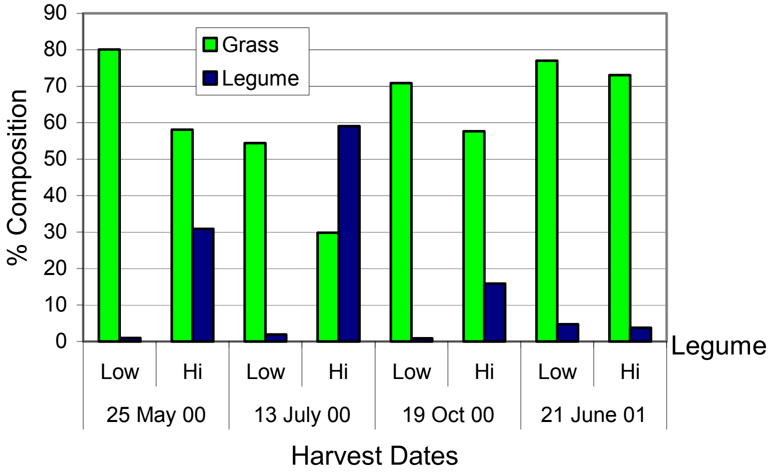

Figure 5. Grass and legume composition as affected by harvest date and level of legume at location 1.
Effect of harvest was significant at *P*<0.001.

A significant harvest x legume interaction was observed when comparing percentage legume at harvest 1 with harvest 4. Legume percentages in the high treatment at the beginning of the experiment (harvest 1) were significantly higher compared to the spring (harvest 4) of the second year (Figure 5, 6 and 7) at all three locations. In contrast, the legume fraction in the low legume stand increased in the second year. This observation was consistent for all three locations. At location 1, low legume stand (1%)

increased to 5% while high legume stand (31%) decreased to 4%. At location 2, low legume stand (2%) increased to 13% while high legume stand (32%) decreased to 15%. At location 3, low legume stand (6%) increased to 31% while high legume stand (36%) decreased to 8%. This can be partly attributed to changes in N status of the soil brought about by the alternate dominance of grass and legume. As explained by Loiseau et al. (2001), higher stands of legumes in the first year may increase available soil N, which favored grasses more than legumes in the following year, resulting in an increase in the grass fraction in the second year. In contrast, low legume and high grass stands in the first year may reduce available soil N, favoring legumes in the following year.

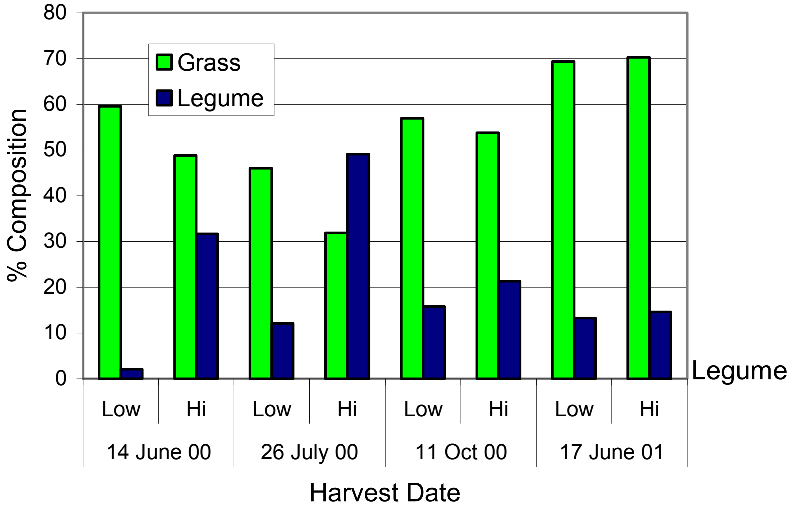

Figure 6. Grass and legume composition as affected by harvest date and level of legume at location 2.
Effect of harvest was significant at *P*<0.001.

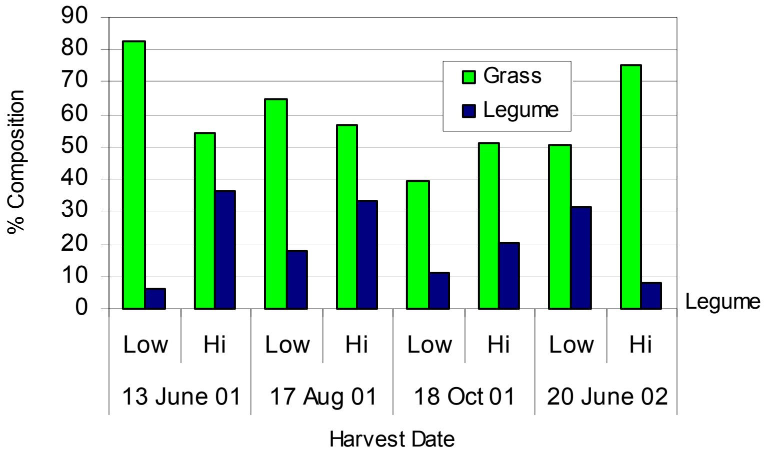

Figure 7. Grass and legume composition as affected by harvest date and level of legume at location 3.
Effect of harvest was significant at $P<0.001$.

Crude Protein

The mean full season crude protein concentrations of the grass fraction in low legume stands were, 119.7, 129.1, and 142.6 g kg^{-1} at locations 1, 2 and 3, respectively. For grass grown in high legume stand these values were 129.7, 139.3 and 150.9 g kg^{-1} at locations 1, 2 and 3, respectively. A significant legume effect was observed only at location three (Table 12, Appendix Tables L, M and N). Crude protein concentration in grass components in high legume stands were generally higher at all harvests at all locations. Averaged over three harvests, grass in the high legume stand had about 7, 8, and 12% more crude protein than grass grown in the low legume stand at locations 1, 2, and 3, respectively.

Table 12. Significance of effects of legume and N on crude protein concentration of the grass fraction.

	Location		
	Morgantown 2000	Reedsville 2000	Reedsville 2001
Legume	NS	NS	**
N	**	*	NS
Legume x N	NS	NS	NS
Harvest	***	***	***

*, **, *** : *F*-tests were significant at, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.
NS : Non significant

A highly significant effect of harvest date was observed on the crude protein concentration of the grass fraction. The highest crude protein concentration was observed at harvest 2 for all three locations (Table 13, 14 and 15). Harvest 1 was the lowest and harvest 3 showed an intermediate value between harvest 1 and 2. The same trend was observed at all three locations. The variation in the crude protein concentration of the grass fraction among three harvests can be attributed to the pattern of change in corresponding legume percentage at each harvest (Figures 5, 6 and 7). Legume percentages were low at first harvest, high at second harvest and intermediate at third harvest. The results suggest that the transfer of biologically fixed N from legume to grasses was responsible for this increase in crude protein concentration. The higher crude protein concentration of the grass in the high legume stand may also have been induced by a lower grass to legume ratio. Nitrogen transfer would have been more efficient and more easily detected under such conditions. In mixed swards with a low percentage of legumes, any transfer of biologically fixed N would have been diluted by the high proportion of grass to legume.

Table 13. Effect of N rate, legume content, and harvest on crude protein concentration of the grass fraction at location 1.

N rate, kg ha^{-1}	Harvest					
	1		2		3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	g kg^{-1}					
0	100.6	102.1	139.5	144.5	110.9	120.8
28	105.9	124.1	133.0	152.0	122.7	119.9
56	103.1	127.8	136.8	146.7	125.3	129.1
Legume mean	103.2	118.0	136.4	147.7	119.7	123.3
Harvest mean	110.6		142.1		121.5	

Effect of N was significant at $P<0.01$.

Table 14. Effect of N rate, legume content, and harvest on crude protein concentration of the grass fraction at location 2.

N Rate, kg ha^{-1}	Harvest					
	1		2		3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	g kg^{-1}					
0	90.9	97.0	137.7	155.7	140.2	152.6
28	103.4	113.7	148.2	154.5	148.8	145.8
56 (Split application)	93.4	112.5	147.9	169.2	151.5	153.1
Legume mean	95.9	107.4	144.6	159.8	146.8	150.5
Harvest mean	101.8		152.2		148.7	

Effect of N was significant at $P<0.05$.

Table 15. Effect of N rate, legume content, and harvest on crude protein concentration of the grass fraction at location 3.

N Rate, kg ha^{-1}	Harvest					
	1		2		3	
	Legume %					
	Low	Hi	Low	Hi	Low	Hi
	g kg^{-1}					
0	91.0	114.5	171.9	173.7	141.0	166.1
28	87.7	111.0	172.1	185.2	146.2	143.1
56 (Split application)	85.4	110.4	169.2	198.4	143.9	156.0
Legume mean	88.1	112.0	171.1	185.8	143.7	155.1
Harvest mean	100.1		178.4		149.4	

Effect of legume was significant at *P*<0.01.

Even though the legume effect on grass crude protein concentration was significant only at one location in this study, previous studies have shown that legume may increase the crude protein concentration of associated grasses (Mallarino et al., 1990; Weller and Cooper, 2001). Herbage samples from only two blocks were analyzed for crude protein concentrations. This may have lowered the possibility of detecting treatment effects. However, Panditaratne et al., (1986) showed that the effect of legume on crude protein concentration of grass can not be detected in mix swards with low (<8%) red clover stands.

The effect of N fertilizer on crude protein concentration of the grass fraction was significant at locations 1 and 2 (Table 12 and Appendix Tables L, M, and N). An increase in crude protein concentration of associated grass was observed with increasing N rates for both high and low legume stands (Figures 8 and 9). However, when orthogonal contrasts were performed for N vs. no N and 28 kg N ha^{-1} vs. 56 kg N ha^{-1} (split in two) the latter was not significant, indicating that only the spring-applied N had an effect on grass crude protein concentration while summer-applied N had no impact. Inefficient recovery of summer applied N fertilizer by grasses was also reported by Stout and Jung (1992).

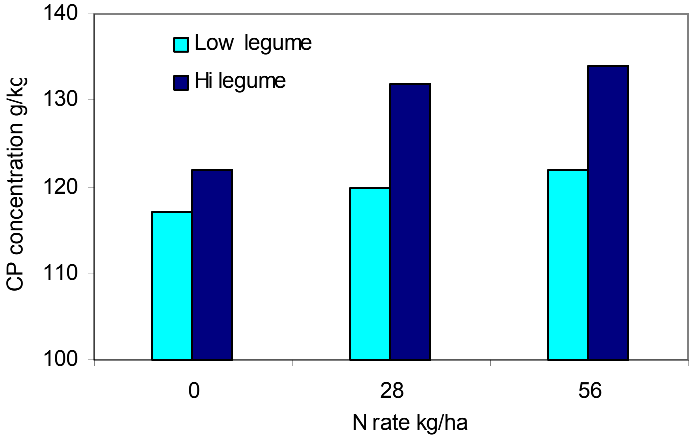

Figure 8. Crude protein concentration of the grass fraction as affected by N at location 1. Effect of N was significant at $P < 0.01$.

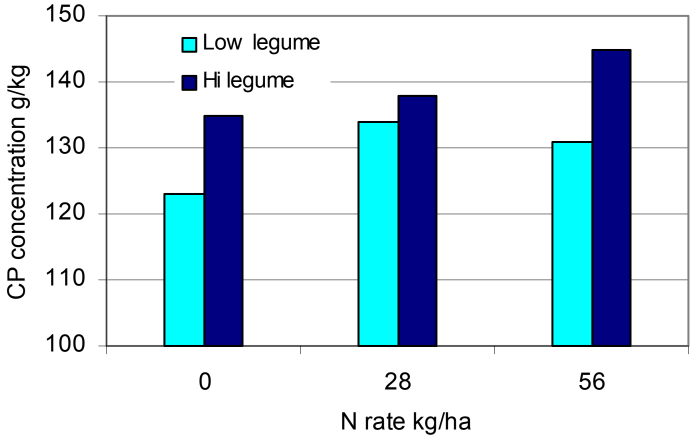

Figure 9. Crude protein concentration of the grass fraction as affected by N at location 2. Effect of N was significant at $P < 0.05$.

The legume fraction in the high legume stand at location 2 was analyzed for crude protein concentration. The results indicated that neither the effect of N nor harvest or the N x harvest interaction were significant (Appendix table O). The average crude protein concentrations of the legume fraction were 174.6, 189.0, and 194.8 g kg^{-1} at harvest 1, 2, and 3, respectively.

CONCLUSIONS

The effect of percentage legume in the sward on herbage accumulation showed consistent results at all three locations. The significant legume effect indicates that herbage accumulation can be increased up to 70% by maintaining a 35% legume stand compared to a low (0-5%) legume stand. Herbage accumulation was not affected by N fertilization. It was shown that application of 28 or 56 (split) kg N ha^{-1} did not result in any significant yield difference in low or high legume stands. The highest yield obtained in a sward with a low percentage legume with N was still lower than the yield in the high legume mixtures without N. It can be concluded that a 25 to 35% red clover stand would be more than sufficient to replace 56 kg of N fertilizer.

The results indicate that it is not economic to apply N at these rates as far as if herbage yield is concerned. However, the significant effect of N fertilizer on grass crude protein concentration suggests that low amounts of N fertilizer can be used when herbage quality is the primary concern. A negative relationship between N fertilizer rate and legume percentage was observed at location 2. Thus, the effect of N on legume persistence has to be considered when applying N fertilizers to mixed swards. In addition, economic and environmental factors have to be considered when making decisions on N fertilizer application.

Treatment effects on botanical composition and crude protein concentration were not consistent at all locations. This variation would be expected due to differences in physical, chemical and biological soil properties, climatic conditions and species composition at different locations.

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APPENDIX

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Figure

Table A. Phosphorous and K fertilizer application in spring 2000 and 2001.

Location	Block	Main Plot	P	K
			kg ha^{-1}	
1	1 to 4	1	0	0
		2	0	0
2	1 to 4	1	27	35
		2	27	35
	5 to 8	1	4	0
		2	4	0
3	1 to 4	1	0	0
		2	50	0
	5 to 6	1	50	0
		2	50	0

Table B. Soil test results in fall 2000 and 2001.

Location	Block	Main Plot	pH	Available			
				P	K	Mg	Ca
				kg ha^{-1}			
1	1 to 4	1	5.8	47	332	262	4411
		2	5.3	58	311	233	2567
2	1 to 4	1	5.8	53	272	323	3557
		2	6.1	64	198	330	4135
	5 to 8	1	5.7	37	189	272	3258
		2	5.7	49	189	337	3810
3	1 to 4	1	5.6	127	415	220	3480
		2	5.4	168	426	237	3747
	5 to 6	1	5.6	81	297	176	3734
		2	5.7	53	273	121	4099

Table C. Analysis of variance of total herbage accumulation for location 1.

Source	df	SS	P
Block	3	8414403	0.017
Legume	1	157428583	0.002
Error a	3	4494330	0.092
N	2	582259	0.604
No-N vs. N	1	14939	0.872
28 vs. 56 kg N	1	567319	0.332
Legume x N	2	69714	0.939
Error b	12	6660783	----

Table D. Analysis of variance of total herbage accumulation for location 2.

Source	df	SS	P
Block	7	35097099	0.001
Legume	1	10383171	0.046
Error a	7	12471275	0.159
N	2	1228369	0.570
No-N vs. N	1	160524	0.701
28 vs. 56 kg N	1	1067844	0.326
Legume x N	2	1845481	0.433
Error b	28	30020625	----

Table E. Analysis of variance of total herbage accumulation for location 3.

Source	df	SS	P
Block	5	12626545	0.068
Legume	1	28225168	0.001
Error a	5	3387134	0.659
N	2	1791517	0.434
No-N vs. N	1	1665086	0.218
28 vs. 56 kg N	1	126430	0.729
Legume x N	2	6928034	0.055
Error b	20	2058336	----

Table F. Analysis of variance of herbage accumulation for location 1.

Source	df	SS	P
Block	3	2804814	0.003
Legume	1	52476187	0.002
Error a	3	1498110	0.047
N	2	194087	0.604
Legume x N	2	23237	0.939
Error b	12	2220260	0.407
Harvest	2	173423286	0.0001
Har. x Leg.	2	11872208	0.0001
Har. x N	4	92211	0.968
Har. x Leg. x N	4	416348	0.661
Error c	36	6189137	----

Table G. Analysis of variance of herbage accumulation for location 2.

Source	df	SS	P
Block	7	11698938	0.0005
Legume	1	3461140	0.046
Error a	7	4157100	0.182
N	2	409428	0.570
Legume x N	2	615207	0.433
Error b	28	10006930	0.618
Harvest	2	217949072	0.0001
Har. x Leg.	2	2279893	0.063
Har. x N	4	2458392	0.198
Har. x Leg. x N	4	1146985	0.581
Error c	84	33533224	----

Table H. Analysis of variance of herbage accumulation for location 3.

Source	df	SS	P
Block	5	3615584	0.036
Legume	1	15824798	0.002
Error a	5	2481790	0.0.135
N	2	1122440	0.275
Legume x N	2	2092936	0.102
Error b	20	8164615	0.137
Harvest	2	469082398	0.0001
Har. x Leg.	2	5278202	0.0003
Har. x N	4	2929658	0.045
Har. x Leg. x N	4	4572642	0.0005
Error c	60	16956372	----

Table I. Analysis of variance of grass and legume components for location 1.

Source	Grass			Legume		
	df	SS	P	df	SS	P
Block	3	138655	0.002	3	67805	0.001
Legume	1	717442	0.068	1	2080290	0.002
Error a	3	278208	0.0001	3	60773	0.001
N	2	30349	0.591	2	12716	0.463
Legume x N	2	39917	0.505	2	6360	0.672
Error b	12	331753	0.001	12	93013	0.025
Harvest	2	994773	0.0001	2	600825	0.0001
Har. x Leg.	2	42772	0.082	2	546616	0.0001
Har. x N	4	33841	0.391	4	5054	0.823
Har. x Leg. x N	4	5813	0.946	4	9586	0.586
Error c	36	287665	----	36	120488	----

Table J. Analysis of variance of grass and legume components for location 2.

Source	Grass			Legume		
	df	SS	P	df	SS	P
Block	7	475187	0.0001	7	147402	0.022
Legume	1	315010	0.019	1	2083908	0.0005
Error a	7	243987	0.005	7	400219	0.0001
N	2	103917	0.009	2	105606	0.029
Legume x N	2	5742	0.738	2	2484	0.910
Error b	28	262441	0.693	28	367864	0.064
Harvest	2	806859	0.0001	2	539276	0.0001
Har. x Leg.	2	78054	0.034	2	648768	0.0001
Har. x N	4	33937	0.554	4	24591	0.576
Har. x Leg. x N	4	20661	0.763	4	19441	0.682
Error c	84	938360	----	84	711019	----

Table K. Analysis of variance of grass and legume components for location 3.

Source	Grass			Legume		
	df	SS	P	df	SS	P
Block	5	376476	0.0001	5	93001	0.092
Legume	1	172776	0.106	1	891002	0.005
Error a	5	222667	0.002	5	204008	0.001
N	2	37566	0.418	2	20730	0.541
Legume x N	2	92177	0.133	2	60135	0.185
Error b	20	412696	0.020	20	327185	0.048
Harvest	2	994819	0.0001	2	183353	0.0002
Har. x Leg.	2	716051	0.0001	2	213200	0.0001
Har. x N	4	43042	0.392	4	23344	0.645
Har. x Leg. x N	4	51994	0.295	4	78311	0.091
Error c	60	618171	----	60	599112	----

Table L. Analysis of variance of crude protein concentration of grass fraction for location 1.

Source	df	SS	P
Block	1	72	0.302
Legume	1	883	0.102
Error a	1	23	0.551
N	2	429	0.009
No-N vs. N	1	448	0.020
28 vs. 56 kg N	1	21	0.569
Legume x N	2	92	0.127
Error b	4	51	0.930
Harvest	2	6128	0.0001
Har. x Leg.	2	197	0.245
Har. x N	4	306	0.350
Har. x Leg. x N	4	376	0.260
Error c	12	749	---

Table M. Analysis of variance of crude protein concentration of grass fraction for location 2.

Source	df	SS	P
Block	1	86	0.344
Legume	1	941	0.494
Error a	1	911	0.007
N	2	514	0.042
No-N vs. N	1	486	0.038
28 vs. 56 kg N	1	28	0.585
Legume x N	2	152	0.220
Error b	4	134	0.821
Harvest	2	18990	0.0001
Har. x Leg.	2	210	0.341
Har. x N	4	283	0.553
Har. x Leg. x N	4	186	0.723
Error c	12	1075	----

Table N. Analysis of variance of crude protein concentration of grass fraction for location 3.

Source	df	SS	P
Block	1	4.2	0.894
Legume	1	2492	0.009
Error a	1	0.4	0.963
N	2	58	0.717
No-N vs. N	1	2.8	0.913
28 vs. 56 kg N	1	55	0.631
Legume x N	2	179	0.415
Error b	4	324	0.837
Harvest	2	37678	0.0001
Har. x Leg.	2	251	0.593
Har. x N	4	390	0.789
Har. x Leg. x N	4	601	0.636
Error c	12	2768	---

Table O. Analysis of variance of crude protein concentration of legume fraction for location 2.

Source	df	SS	P
Block	1	1190	0.050
N	2	114	0.650
No-N vs. N	1	40	0.669
28 vs. 56 kg N	1	74	0.563
Error a	2	213	0.611
Harvest	2	1299	0.110
Har. x N	4	571	0.611
Error b	6	1198	----

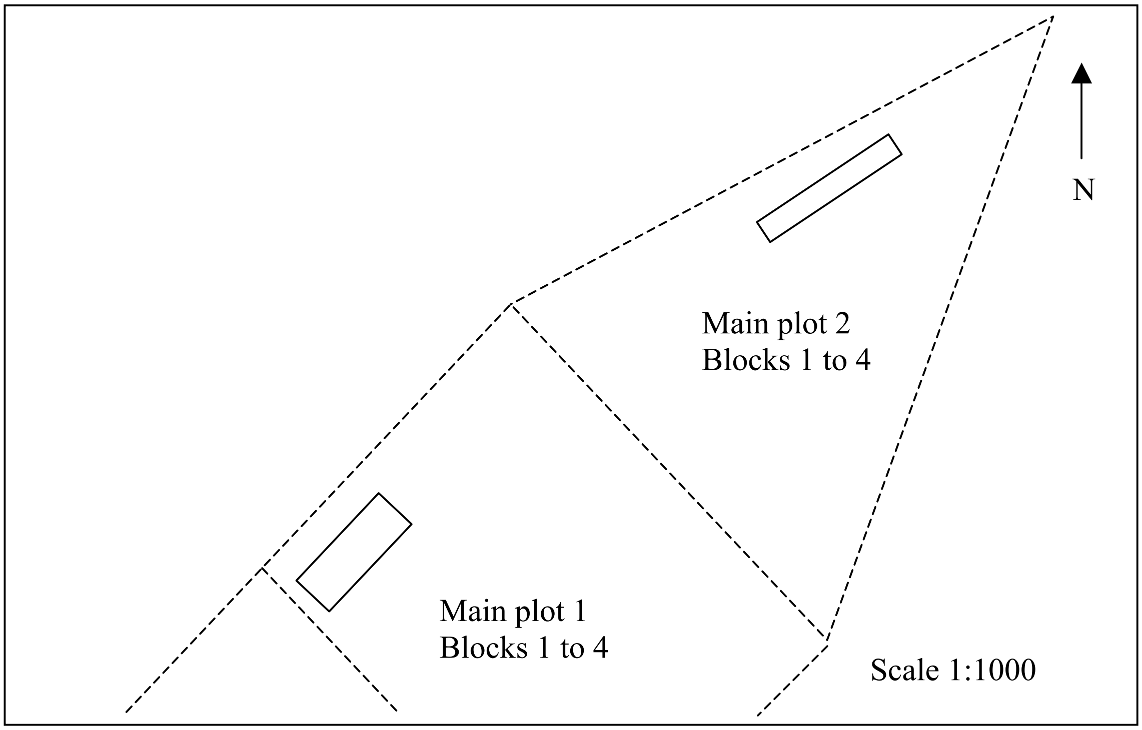

Figure A. Site map of location 1.

------- Plot boundaries of grazing experiment (HOG).

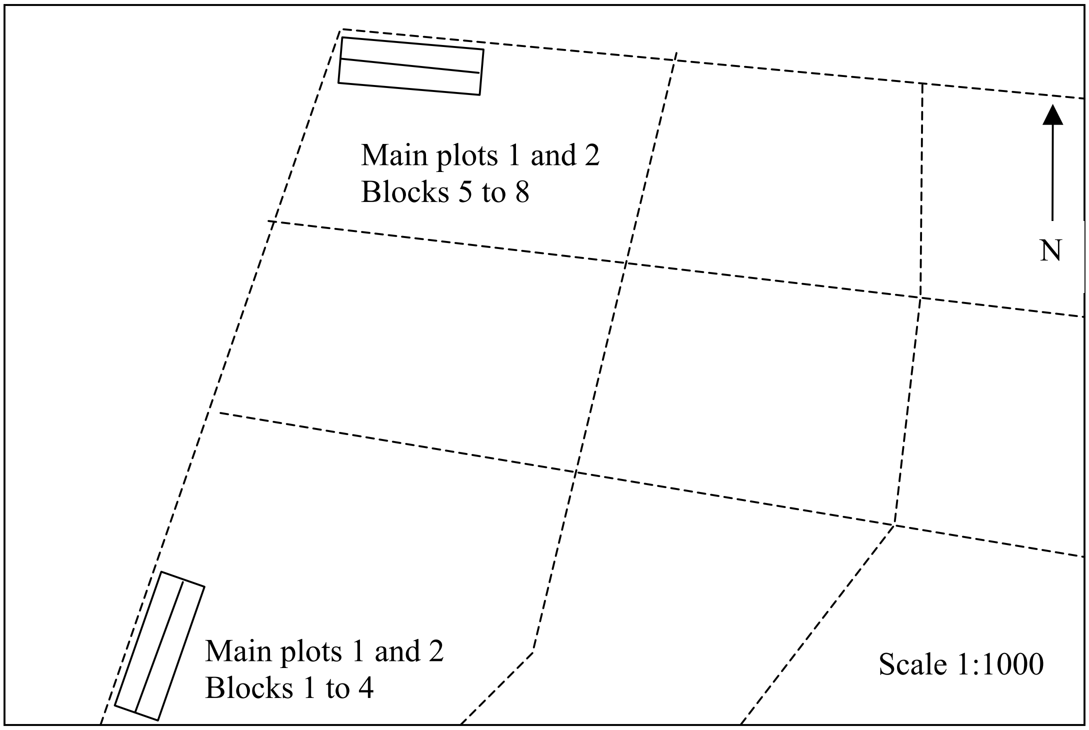

Figure B. Site map of location 2.

------- Plot boundaries of grazing experiment (CCS).

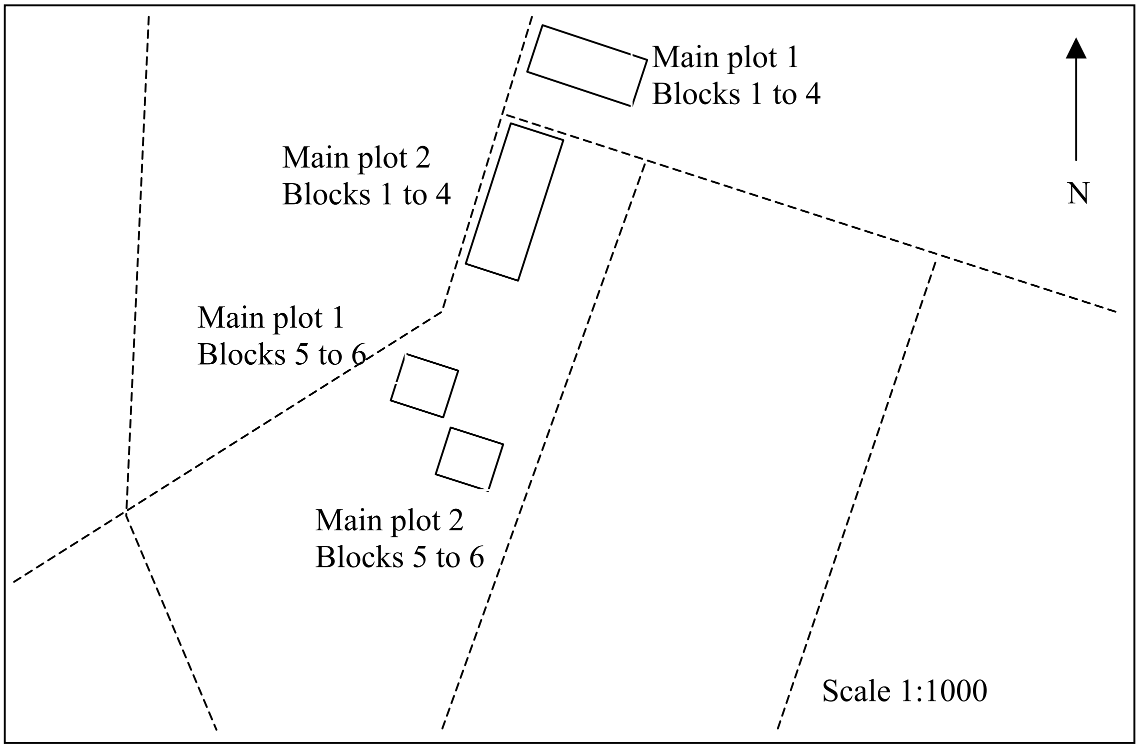

Figure C. Site map of location 3.

------- Plot boundaries of grazing experiment (CCS).

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