I. EFFECT OF NITROGEN RATE ON PLANT NITROGEN LOSS IN WINTER WHEAT VARIETIES

II. GLUTAMINE SYNTHETASE ACTIVITY IN WINTER WHEAT VARIETIES

III. EFFECT OF NITROGEN FERTILIZATION AND CATION REMOVAL ON SOIL pH

By

FRED KABURU KANAMPIU

Bachelor of Science in Agriculture
University of Nairobi
Nairobi, Kenya
1985

Master of Science in Agronomy
University of Nairobi
Nairobi, Kenya
1988

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Graduate College of the
Oklahoma State University
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Thesis Approved:

William Lamb
Thesis Adviser

Gordon W. Johnson

Brett F. Carver

James D. Ownby

Thomas C. Collins
Dean of the Graduate College

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INTRODUCTION

There are three chapters in this dissertation, each discussing the results of research that was conducted for my doctoral degree. Each chapter is presented in a form suitable for publication in a professional journal.

CHAPTER I

EFFECT OF NITROGEN RATE ON PLANT NITROGEN LOSS IN WINTER WHEAT VARIETIES

ABSTRACT

Gaseous nitrogen (N) loss from wheat (*Triticum aestivum* L.) plants has been identified but has not been simultaneously evaluated for several genotypes grown under different N fertility. Two field experiments were initiated in 1993 and 1994 at the Agronomy Research Station in Stillwater and Perkins to estimate plant N loss from several cultivars and experimental populations as a function of N applied and to characterize N use efficiency (NUE-grain weight (Gw) per unit area/N supply (Ns) per unit area) as affected by time of N fertilization. A total of 5 cultivars and 3 experimental populations were evaluated at preplant N rates ranging from 30 to 180 kg ha^{-1}. Nitrogen loss was estimated as the difference between total forage N at anthesis and the total (grain + straw) N at harvest. Forage, grain, and straw yield and N uptake, and N loss increased with increasing N applied at both Stillwater and Perkins. Significant differences were observed among the entries for yields, N uptake, N loss and components of

NUE in forage, grain, straw and grain + straw. Estimates of N loss over this two year period ranged from 4.0 to 27.9 kg ha^{-1} (7.1 to 37.2 % of total forage N at anthesis). Most N losses occurred between anthesis and fourteen days post-anthesis. Avoiding excess N application would reduce N losses and hence increase NUE in winter wheat varieties. Entries with high harvest index and low forage yield had low plant N loss. Estimates of plant loss suggest N balance studies should consider this variable before assuming that all unaccounted N was lost to leaching and denitrification.

INTRODUCTION

Worldwide interest associated with increasing cereal grain protein has focused added attention on improving the utilization of N in cereals (Desai and Bhatia, 1978). The effectiveness with which N is used by wheat and other cereals has become increasingly important because of increased costs associated with the manufacture and distribution of N fertilizer. Nitrogen is an essential element for plant growth, plays a crucial role in crop production, and is usually the most costly fertilizer input used to produce non-legume crops. Increased use of fertilizer N in agricultural production has raised concerns because of the potential for groundwater contamination. Subsequently, this has placed pressure on farmers to use N more efficiently.

Nitrogen use efficiency is defined as grain production per unit of N available in the soil (Moll *et al.*, 1982; Van Sanford and Mackown, 1986). Nitrogen uptake and partitioning between straw and grain are the two major components of N economy in plants (Desai and Bhatia, 1978). Partitioning N between grain and straw is important in crops like wheat which are extensively grown in areas where plants face depleted soil N and moisture during the grain filling period. Uptake efficiency (total shoot N/soil N supply) and utilization (grain yield/total shoot N) of N in the production of grain requires that the processes of uptake, translocation, assimilation, and redistribution of N operate effectively. The relative contribution of these processes to genotypic differences in NUE is unknown and varies among genetic populations and among environments, including N supply. Moll *et al.* (1982) observed an interaction between corn hybrids and N levels for all traits except grain yield. At low N supply, differences among hybrids for NUE were largely due to variation in utilization of accumulated N, but with high N they were largely due to variation in uptake efficiency. They concluded that variation of NUE appeared to result from differences among genotypes and levels of N supplied.

Wuest and Cassman (1992) found recovery of N applied at planting ranged from 30 to 55 %, while recovery of N applied at anthesis ranged from 55 to 80 % in an irrigated wheat. The amount of fertilizer N applied at anthesis had the greatest influence on post-anthesis N uptake, which ranged from 17 to 77 kg N ha^{-1}. This shows that late N application can be efficiently taken up by plants.

Grain protein levels may increase with late-season N applications (Wuest and Cassman, 1992). Fertilizer N use efficiency varies considerably depending upon the native soil N supply, previous N uptake, developmental stage of the plant when supplemental N is applied, and yield potential (Wuest and Cassman, 1992). Optimizing fertilizer N use and at the same time achieving acceptable yield levels and adequate grain protein requires knowledge of expected N uptake efficiency and utilization within the plant in relation to the rate and timing of N applied.

Calculations for N fertilizer use efficiencies are typically based on the amount of N found in the crop at maturity. It is commonly perceived that maximum accumulation of N by plants occurs at maturity; however, it is more typical for maximum N accumulation of grain crops to be reached sometime between pollination and maturity (Francis, 1993a). Dhugga and Waines (1989) found differences among wheat genotypes for shoot N accumulation before and after anthesis at the highest soil N level. At this level, some genotypes either stopped accumulating or showed a net loss of shoot N between anthesis and maturity, which appeared to be associated with superior preanthesis N accumulation capacity and reduced grain N yield of such genotypes.

Plant shoots may be a significant source of N loss in crops. Volatile N has been found to be released from plant tissue, NH_3 being the prevalent form of post-anthesis N loss (Harper *et al.*, 1987). Francis *et al.* (1993b) found maximum net N accumulation in corn to occur during early reproductive

development (R1 - R3) followed by a subsequent decline. They found plant N loss could account for 52 to 73 % of the unaccounted N in ^{15}N balance calculations. Ammonia loss rates on a leaf-area basis from wheat were found to be similar for low and high N plants despite significantly high N concentrations in high N plants (Parton *et al.*, 1988). They found twice the leaf area was attained by the high N plants, resulting in NH_3 volatilization rates per plant roughly twice those observed in the low-N plants. Nitrogen loss from wheat plants through aerial NH_3 transport has also been found during periods when there is adequate available soil N (Harper *et al.*, 1987) and during plant senescence (Harper *et al.*, 1987; Parton *et al.*, 1988). Harper *et al.* (1987) found largest aerial loss to occur during a 20-day period after fertilizer application (11.4 % of the applied fertilizer) while additional losses (9.8 %) were observed from anthesis to harvest. The former aerial NH_3 losses could have been due to overloading of plant N as NH_4^+ whereas the latter could be due to plant senescence and inefficient redistribution of N within the plant. High N fertility levels often increase leaf area indices, but the greatest difference during maturation is the ability to maintain a larger number of green leaves late in the season as compared with low N fertility levels. Plant N losses could account for much of the N losses found in soil N balance studies and certainly influence calculations involving fertilizer N efficiency (Daigger *et al.,* 1976). Failure to include direct plant N losses when calculating an N budget can lead to overestimation of losses from the soil by denitrification, leaching, and ammonia volatilization (Francis *et al.*, 1993b). Proper accounting

for volatile plant N losses may play an important role in developing cropping systems that have improved N fertilizer use efficiencies and reduced environmental impact.

Remobilization of vegetative N during grain fill in wheat contributes significantly to final grain N content. Van Sanford and Macknown (1987), working with soft red winter wheat, detected significant cultivar differences in N remobilization from the flag leaf, peduncle, and lower culm. The proportion of N accumulated by the spike ranged among cultivars from 51 to 91 %. They also found 83 % of the total above ground N at maturity to be present in the plant at anthesis. An analysis of cultivar differences indicated that all of the cultivar variation in final spike N could be associated with variation in total N uptake. Higher post-anthesis N uptake was associated with lower N utilization efficiency (spike wt/total plant N), higher grain N concentration, and lower grain yields (Van Sanford and Macknown, 1987).

Although soil fertility research programs have been successful in establishing fertilizer N optimums for selected wheat varieties, little work has been done to improve genetic NUE in wheat. Therefore, plant breeders need to develop cultivars that can absorb N more efficiently from the soil and effectively partition absorbed N to the grain. Such cultivars could minimize loss of N from the soil and make more economic use of the absorbed N (Dhugga and Waines, 1989). Because crop fertilizer recovery seldom exceeds 50 %, the potential for increasing NUE has stimulated new research. It is the unaccounted portion in

the crop that is currently being addressed in research. Effective use of applied N by the crop will reduce input costs per unit of product harvested. Identification of N use efficient wheat varieties could decrease N fertilizer requirements and limit the potential for NO_3-N leaching losses. More studies are required to identify wheat varieties which maintain high yield potential with lower N fertilizer requirements.

The objective of this research was to estimate plant N loss from several wheat cultivars and experimental populations as a function of N applied and to characterize NUE as affected by time of N fertilization.

MATERIALS AND METHODS

Two field experiments were initiated in October 1993 and 1994 at the Agronomy Research Station in Stillwater and Perkins to estimate plant N loss from several wheat cultivars and experimental populations as a function of N applied, and to characterize NUE as affected by time of N fertilization. A total of 4 wheat cultivars (Karl, 2180, TAM W-101, and Chisholm) and 3 experimental populations were evaluated. The experimental populations consisted of an unselected hard winter wheat population (Control Composite) and the same population subjected to two cycles of selection for either high or low yield potential under sub-optimal N application. The cultivar Longhorn was included in the Perkins experiment in addition to the others included at Stillwater. All

cultivars and experimental populations were evaluated at preplant N rates of 0, 30, 60 and 120 kg ha^{-1} (Stillwater) and 0, 45, 90 and 180 kg ha^{-1} (Perkins). Urea ammonium nitrate (UAN, 28-0-0) was used as the N source applied at planting for all N treatments except for three additional N treatments applied as a split in the Perkins experiment. A complete factorial arrangement of treatments was used (N rate x genotype) in a randomized complete block experimental design with four and three replications for Stillwater and Perkins, respectively. Soil classification, initial soil characteristics, plot size, harvest areas and harvest dates are reported for Stillwater and Perkins in Tables 1 and 2. Sufficient area was available in each plot to accommodate forage harvest and grain yield in separate areas of each plot. Forage harvests were obtained by hand clipping all plants 2 cm above ground at anthesis. Subsamples from each respective harvest were collected for moisture and total N analysis. All forage and grain samples were ground in a large Wiley mill and later in an automated grinding unit to obtain finely ground forage, grain and straw subsamples. Total N was determined on forage, grain and straw samples using a Carlo-Erba NA 1500 dry combustion analyzer (Schepers *et al.*, 1989). Nitrogen use efficiency was analyzed according to an expanded model of Moll *et al.* (1982). Nitrogen use efficiency for grain yield was partitioned into various components as follows:

Gw/Ns = grain weight/N supply (applied N to the plant)'

Gw/Ns = (Nt/Ns)(Gw/Nt), where

Nt/Ns = uptake efficiency = ratio of total plant to N supply per unit area,

Nt = (grain yield)(grain N) + (dry wt of stem and leaves)(N in stem and leaves),

Gw/Nt = utilization efficiency = (Gw/Ng)(Ng/Nt), where

Gw/Ng = grain weight/grain N and

Ng/Nt = translocation efficiency = proportion of total plant N in the grain. Nitrogen loss was estimated as the difference between total forage N at anthesis and the total (grain + straw) N at harvest. Data analysis was performed using SAS (SAS Institute Inc., 1988). Means were compared using Student-Newman-Keuls' (S-N-K) test at the 5% significance level.

RESULTS AND DISCUSSION

At both locations, forage, grain and straw yield, and forage, grain, straw and grain + straw N uptake increased with increasing N applied (Tables 3 and 4). The exception to this was noted for straw and forage yield at Stillwater and Perkins, respectively. Interpretation of N rate and entry main effects was simplified at Stillwater since no N rate by entry interactions were found for any of the measured dependent variables (Table 3). At Perkins a highly significant N rate by entry interaction was found for grain and straw yield, and straw and grain + straw N uptake, thus restricting interpretation of main effect means (Table 4). At both locations there were differences among entries for forage, grain and straw yield and forage, grain, straw and grain + straw N uptake. The cultivars Chisholm and TAM W-101 both had high yield and N uptake in forage, grain and

grain + straw compared with other entries at Stillwater. At Perkins, Chisholm, Karl, 2180 and Longhorn (which was not included at Stillwater) had high yield and N uptake in forage and grain.

Excluding NUE at Stillwater and NUE and N uptake efficiency at Perkins no N rate by entry interactions were found for N use efficiency variables (Tables 5 and 6). Increased applied N generally decreased NUE, N uptake efficiency, N utilization efficiency, fraction of N translocated to grain and grain yield per grain N, but increased protein content and N loss (Tables 5 and 6). However, the increase in fraction of N translocated to the grain and increased N loss with increased applied N at Perkins was not significant. Generally, percent protein and N loss were lower at Stillwater when compared to Perkins. The opposite was observed for other NUE components. Nitrogen loss ranged from 4.0 to 26.3 and 11.3 to 27.9 kg ha^{-1} (averaged over N rates) at Stillwater and Perkins, respectively. In terms of the proportion of N accumulated in the plants at anthesis, N loss ranged from 7.1 to 37.0 % and 25.1 to 37.2 % at Stillwater and Perkins, respectively. Similar results of N loss from wheat plants through aerial NH_3 transport have also been found during periods when there is adequate available soil N (Harper *et al.*, 1987).

Except for percent protein and grain yield per grain N at Stillwater and N loss at Perkins, the entries evaluated showed differences in NUE components at both locations (Tables 5 and 6). At Stillwater, Chisholm and TAM W-101 had higher NUE, N uptake efficiency and N utilization efficiency whereas at Perkins

2180 and Longhorn had higher N use and N uptake efficiency compared to other entries evaluated. These results agree with the work of Daigger *et al.* (1976) and Dhugga and Waines (1989) who found differences among wheat genotypes for shoot N accumulation before and after anthesis. Differences between entries were also found at various N rates for grain and straw yield, and straw and grain + straw N uptake, N use and N uptake efficiency at Perkins (Table 7). Similar differences were found for NUE at Stillwater. All evaluated entries showed a decrease in N uptake between anthesis and maturity at Perkins (Figure 1). Longhorn and 2180 had the highest N loss while Control Composite had the lowest. The loss was greater between anthesis and fourteen days post-anthesis as compared to fourteen days post-anthesis and maturity. This suggests most N losses occurred prior to and early in the grain filling period when N is rapidly translocated from other plant parts to the head. During anthesis, protein in stems and leaves is degraded to its constituent amino acids and/or NH_3. Ammonia assimilation occurs to incorporate the released N into amino acids. Depending on various factors such as temperature, light, wind, moisture, pH among others, NH_3 formed during protein degradation can be lost from the plant by volatilization. At Perkins, it was interesting to observe that N loss from anthesis to fourteen days post-anthesis was high for Logrp at the low N rates (0, 45 kg ha^{-1}) and low at the higher N rates (90, 180 kg ha^{-1}). The opposite of this was found for Higrp. Results from response surface modeling suggest that N loss increases with increasing forage yield and percent forage N (Figures 2 and

3). This indirectly suggests that cultivars with a high harvest index and low forage yields will have low plant N loss. Estimates of plant N loss in this work also suggest that N balance studies should consider this variable before assuming that all unaccounted N was lost to leaching or denitrification.

Split application of 180 kg N ha^{-1} decreased forage, grain and straw yield, and forage, grain and grain + straw N uptake for Longhorn (Table 8). However, split application of N had no effect on forage, grain, straw and grain + straw yield and N uptake for TAM W-101 (Table 8). Split application of 90 kg N ha^{-1} also decreased straw yield and straw and grain + straw N uptake for Longhorn. Split application of 180 kg N ha^{-1} decreased grain yield/grain N and N loss for Longhorn only (Table 9). These results tend to suggest that split application of N could be one way of reducing N loss and hence increasing NUE in winter wheat.

It is important to note that estimates of plant N loss in this work have likely been underestimated since soil N uptake and plant N loss are dynamic processes which always occur as the plant grows towards maturity. This is because our work did not identify the exact date (physiological stage) where N accumulated in wheat was at maximum. Based on the literature cited, flowering was the best estimate (Daigger *et al.*, 1976). In addition, plant N loss as has been estimated here assumes that no added soil N uptake took place beyond flowering. This is somewhat unrealistic since we know that the wheat plant continues to assimilate soil N beyond flowering (Harper et al., 1987). Therefore

continued plant loss of additional assimilated soil N (beyond flowering) would not be accounted for using our methods.

CONCLUSIONS

Forage, grain and straw total yield and N uptake, and N loss were significantly increased with increasing N applied.

Nitrogen loss ranged from 4.0 to 26.3 and 11.2 to 27.9 kg ha^{-1} (averaged over N rates) at Stillwater and Perkins, respectively. Avoiding excess N application could reduce N losses and hence increase NUE in winter wheat varieties. Estimates of plant N loss from anthesis to fourteen days post-anthesis were greater than that from fourteen days post-anthesis to maturity. Results from response surface modeling suggest that N loss increased with increasing forage yield and percent forage N. This indirectly indicates that entries with a high harvest index and low forage yield will have low plant N loss. Estimates of plant loss in this work suggest N balance studies should consider this variable before assuming that all unaccounted N was lost to leaching and denitrification. Decreased N loss with split application of N was beneficial in increasing NUE in winter wheat varieties.

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Table 1. Soil chemical characteristics and classification at Stillwater and Perkins, OK.

Location	pH	NH_4-N	NO_3-N	P	K	N	Organic C
			mg kg^{-1}				g kg^{-1}
Stillwater	5.5	10.2	5.5	38	20.9	0.67	6.4
Classification: Kirkland silt loam (fine-mixed, thermic Udertic Paleustoll)							
Perkins	6.0	19.1	6.5	11.8	29.5	0.66	7.4
Classification: Teller sandy loam (fine-loamy, mixed, thermic Udic Argiustoll)							

pH - 1:1 soil:water, K and P - Mehlich III, Organic C and Total N - dry combustion.

Table 2. Soil chemical characteristics and classification at Stillwater and Perkins, OK.

	Stillwater	Perkins
Plot size:	1.125 x 15.2 m	1.125 x 15.2 m
Number of rows:	5	5
Planting date:	October 27, 1993	October 24, 1994
Forage at anthesis:		
harvest area	0.9144 x 4.6 m	0.45 x 3 m
harvest date	May 12th, 1994	April 24th, 1995
Forage at post-anthesis:		
harvest area	NA	0.45 x 3 m
harvest date	NA	May 8th, 1995
Grain:		
harvest area:	1.143 x 10.6 m	1.125 x 9 m
harvest date:	June 20th, 1994	June 14th, 1995
Straw:		
harvest area:	1.143 x 10.6 m	1.125 x 9 m
harvest date:	June 20th, 1994	July 14th, 1995

Table 3. Analysis of variance, means and comparisons for yield and nitrogen uptake, Stillwater, OK 1994.

		Forage	Grain	Straw	Forage	Grain	Straw	(Grain + Straw)
		yield (Mg ha^{-1})			nitrogen uptake (Mg ha^{-1})			
Source of variation	df	mean squares						
Replication	3	21.2**	0.3ns	0.1ns	0.008**	0.0002ns	0.0002**	0.0008*
N rate	3	11.1**	1.8**	0.3ns	0.007**	0.002**	0.00008*	0.002**
Entry	6	4.2**	1.6**	0.5*	0.0008*	0.0007**	0.00005*	0.0005**
N rate * entry	18	0.6ns	0.1ns	0.2ns	0.0003ns	0.00005ns	0.00001ns	0.00007ns
Residual	81	0.7	0.1	0.2	0.0003	0.00007	0.00001	0.00009
					means, Mg ha^{-1}			
N rate, kg ha^{-1}:								
0		3.33	1.37	1.91	0.049	0.029	0.011	0.040
30		3.89	1.67	1.66	0.059	0.038	0.010	0.047
60		4.47	1.87	1.84	0.070	0.044	0.012	0.055
120		4.74	1.93	1.74	0.086	0.047	0.014	0.061
SED		0.22	0.09	0.13	0.004	0.002	0.001	0.003
Entry:								
Chisholm		4.68	2.23	1.79	0.073	0.049	0.010	0.059
Composite		4.18	1.45	1.62	0.071	0.033	0.011	0.044
Higrp		3.64	1.60	1.75	0.059	0.037	0.012	0.049
Karl		4.06	1.69	1.55	0.066	0.040	0.10	0.050
Logrp		3.84	1.37	1.91	0.063	0.031	0.013	0.044
2180		3.49	1.58	2.10	0.056	0.037	0.015	0.052
TAM W-101		4.85	2.05	1.81	0.074	0.047	0.011	0.057
SED		0.29	0.12	0.18	0.060	0.003	0.001	0.003
Contrasts:								
N rate linear	1	**	**	ns	**	**	**	**
N rate quadratic	1	ns	ns	ns	ns	ns	ns	ns

*, ** Significant at 0.05 and 0.01 probability levels, respectively. SED - standard error of the difference between two equally replicated means.

Table 4. Analysis of variance, means and comparisons for yield and nitrogen uptake, Perkins, OK 1995.

Source of variation	df	Forage	Grain	Straw	Forage	Grain	Straw	(Grain + Straw)
		yield (Mg ha^{-1})			nitrogen uptake (Mg ha^{-1})			
		mean squares						
Replication	2	2.5*	0.04ns	0.2ns	0.001*	0.00005ns	0.00007ns	0.0002*
N rate	3	1.1ns	0.2**	0.9**	0.002**	0.0004**	0.0002**	0.001**
Entry	7	3.4**	0.3**	3.7**	0.0009*	0.0002**	0.0003**	0.0004**
N rate * entry	21	0.8ns	0.06*	0.4**	0.0004ns	0.00002ns	0.00005*	0.0001**
Residual	57	0.7	0.03	0.1	0.0003	0.00002	0.00003	0.00006
					means, Mg ha^{-1}			
N rate, kg ha^{-1}:								
0		2.82	0.76	1.51	0.052	0.020	0.016	0.035
45		3.18	0.83	1.49	0.060	0.023	0.016	0.040
90		3.02	0.79	1.54	0.065	0.025	0.018	0.043
180		3.39	0.97	1.93	0.078	0.031	0.023	0.053
SED		0.24	0.05	0.10	0.005	0.001	0.002	0.002
Entry:								
Chisholm		3.31	0.90	1.28	0.063	0.026	0.016	0.041
Composite		2.15	0.62	1.28	0.045	0.019	0.016	0.034
Higrp		2.85	0.78	1.35	0.059	0.024	0.015	0.039
Karl		3.19	0.87	1.17	0.065	0.026	0.012	0.039
Logrp		2.72	0.61	2.13	0.062	0.020	0.024	0.043
2180		3.82	1.07	1.21	0.075	0.032	0.015	0.047
TAM W-101		2.92	0.86	1.68	0.066	0.023	0.018	0.042
Longhorn		3.95	1.02	2.80	0.077	0.027	0.028	0.055
SED		0.35	0.07	0.15	0.008	0.002	0.002	0.003
Contrasts:								
N rate linear	1	ns	**	**	**	**	**	**
N rate quadratic	1	ns	ns	*	ns	ns	ns	ns

*, ** Significant at 0.05 and 0.01 probability levels, respectively. SED - standard error of the difference between two equally replicated means.

Table 5. Analysis of variance, means and comparisons for nitrogen use efficiency components, Stillwater, OK 1994.

		Protein %	N-use efficiency (Gw/Ns)	N-uptake efficiency (Nt/ Ns)	N-utilization efficiency (Gw/Nt)	Fraction of N translocated to grain(Ng/Nt)	Grain yield/ grain N (Gw/Ng)	N loss ($kg\ ha^{-1}$) (Na-Nh)
Source of variation	df	mean squares						
Replication	3	71.2**	81.3ns	0.38**	855.3**	0.03**	910.4**	6162.5**
N rate	3	19.3**	11145.1**	8.24**	61.5**	0.02**	182.7**	1533.7**
Entry	6	1.8ns	574.2**	0.18**	108.3**	0.04**	19.6ns	771.5**
N rate * entry	18	1.0ns	88.1*	0.03ns	11.0ns	0.002ns	10.8ns	340.4ns
Residual	81	1.8	38.2	0.04	14.0	0.004	18.9	224.3
					means			
N rate, $kg\ ha^{-1}$								
0		12.1	0	0	34.7	0.73	47.4	8.8
30		12.9	55.6	1.6	35.6	0.79	45.1	11.8
60		13.1	31.1	0.9	34.6	0.77	44.8	14.6
120		14.1	16.1	0.5	32.2	0.77	41.2	25.7
SED		0.36	1.65	0.05	1.00	0.02	1.16	4.00
Entry:								
Chisholm		12.5a	46.2	1.2	38.2	0.8	46.5a	13.9
Composite		12.9a	28.1	0.8	33.9	0.8	45.0a	26.3
Higrp		13.1a	33.2	1.0	33.9	0.8	44.4a	10.6
Karl		13.4a	34.0	1.0	34.3	0.8	43.1a	15.9
Logrp		13.2a	27.0	0.9	31.6	0.7	44.6a	18.8
2180		13.5a	31.5	1.0	31.1	0.7	43.8a	4.0
TAM W-101		12.9a	39.8	1.1	37.1	0.8	45.3a	17.1
SED		0.47	2.52	0.07	1.32	0.02	1.54	5.30
Contrasts:								
N rate linear		**	**	**	**	*	**	**
N rate quadratic		ns	**	**	*	*	ns	ns

*, ** Significant at 0.05 and 0.01 probability levels, respectively. Gw = grain weight; Ns = N supply; Na = N accumulated in plant at anthesis; Nt = total N in plant at maturity; Ng = N accumulated in grain at harvest; Nh = N accumulated in plant at harvest. SED - standard error of the difference between two equally replicated means.

Table 6. Analysis of variance, means and comparisons for nitrogen use efficiency components, Perkins, OK 1995.

		Protein %	N-use efficiency (Gw/Ns)	N-uptake efficiency (Nt/ Ns)	N-utilization efficiency (Gw/Nt)	Fraction of N translocated to grain(Ng/Nt)	Grain yield/ grain N (Gw/Ng)	N loss ($kg\ ha^{-1}$) (Na-Nh)
Source of variation	df				mean squares			
Replication	2	87.4**	18.4ns	0.03ns	161.**	0.002ns	360**	737.7ns
N rate	3	32.4**	1293.8**	2.4**	59**	0.003ns	164.1**	280.8ns
Entry	7	10.2**	65.5**	0.09**	82**	0.085**	47.7**	282.2ns
N rate * entry	21	0.7ns	20.2**	0.03*	7.5ns	0.007ns	4.1ns	269.4ns
Residual	81	1.1	7.5	0.02	7	0.005	4.9	239.1
					means			
N rate, $kg\ ha^{-1}$								
0		15.1	0	0	22.1	0.57	38.6	17.8
45		16.3	20.6	1.0	21.1	0.60	35.7	20.9
90		17.5	9.9	0.5	19.2	0.58	32.9	22.4
180		17.6	6.1	0.3	19.1	0.58	32.8	27.0
SED		0.32	0.82	0.04	0.80	0.02	0.67	4.66
Entry:								
Chisholm		16.3	11.8	0.5	22.4	0.6	35.3	21.8a
Composite		16.9	9.5	0.5	18.4	0.5	34.1	11.3a
Higrp		17.3	11.0	0.6	20.2	0.6	33.4	19.8a
Karl		17.5	13.1	0.6	23.0	0.7	33.0	26.6a
Logrp		16.7	9.5	0.7	15.0	0.4	35.6	22.2a
2180		17.4	18.1	0.8	22.7	0.7	33.4	27.9a
TAM W-101		15.5	11.7	0.6	21.4	0.6	37.4	24.7a
Longhorn		15.0	14.7	0.8	19.5	0.5	38.5	22.3a
SED		0.45	1.37	0.06	1.13	0.03	0.95	6.59
Contrasts:								
N rate linear		**	**	**	**	ns	**	ns
N rate quadratic		ns	**	**	ns	ns	ns	ns

*, ** Significant at 0.05 and 0.01 probability levels, respectively. Gw = grain weight; Ns = N supply; Na = N accumulated in plant at anthesis; Nt = total N in plant at maturity; Ng = N accumulated in grain at harvest; Nh = N accumulated in plant at harvest. Means followed by same letter are not significantly different at 0.05 probability level. SED - standard error of the difference between two equally replicated means.

Table 7. Means for yield and components of N use efficiency at various nitrogen rates, Perkins, OK 1995.

	Grain yield (Mg ha^{-1})	Straw yield (Mg ha^{-1})	Straw N uptake (Mg ha^{-1})	(Grain + Straw N) (uptake Mg ha^{-1})	N-use efficiency (Gw/Ns)	N-uptake efficiency (Nt/ Ns)
0 kg N ha^{-1}						
Chisholm	0.89	1.47	0.019	0.042	0	0
Composite	0.58	1.34	0.017	0.033	0	0
Higrp	0.70	1.19	0.012	0.032	0	0
Karl	0.86	1.19	0.011	0.035	0	0
Logrp	0.64	2.07	0.022	0.033	0	0
2180	0.64	0.87	0.011	0.030	0	0
TAM W-101	0.87	1.85	0.017	0.038	0	0
Longhorn	0.81	1.89	0.014	0.033	0	0
45 kg N ha^{-1}						
Chisholm	0.93	1.34	0.013	0.040	23	1.0
Composite	0.55	1.12	0.013	0.029	14	0.7
Higrp	0.68	1.25	0.014	0.034	17	0.9
Karl	0.78	0.72	0.001	0.032	19	0.8
Logrp	0.75	2.25	0.024	0.047	19	1.2
2180	1.28	1.49	0.017	0.052	32	1.3
TAM W-101	0.76	1.28	0.016	0.037	19	0.9
Longhorn	0.91	2.46	0.026	0.048	23	1.2
90 kg N ha^{-1}						
Chisholm	0.76	0.85	0.011	0.034	10	0.4
Composite	0.70	1.41	0.017	0.039	9	0.5
Higrp	0.83	1.30	0.014	0.039	10	0.5
Karl	0.91	1.44	0.016	0.044	11	0.5
Logrp	0.49	1.75	0.022	0.041	6	0.5
2180	1.10	1.16	0.014	0.050	14	0.6
TAM W-101	0.79	1.74	0.019	0.042	10	0.5
Longhorn	0.91	3.22	0.035	0.061	11	0.8
180 kg N ha^{-1}						
Chisholm	1.01	1.50	0.018	0.049	6	0.3
Composite	0.64	1.25	0.015	0.035	4	0.2
Higrp	0.93	1.66	0.021	0.052	6	0.3
Karl	0.95	1.44	0.017	0.046	6	0.3
Logrp	0.57	2.59	0.033	0.051	4	0.3
2180	1.13	1.19	0.016	0.051	7	0.3
TAM W-101	1.03	1.83	0.022	0.055	6	0.3
Longhorn	1.41	3.78	0.039	0.078	9	0.5
SED	0.14	0.28	0.004	0.006	2.2	0.1

Gw = grain weight; Ns = N supply; Na = N accumulated in plant at anthesis; Nt = total N in plant at maturity; Ng = N accumulated in grain at harvest; Nh = N accumulated in plant at harvest.

Table 8. Analysis of variance and single degree of freedom contrasts on yield and nitrogen uptake as affected by split application of nitrogen, Perkins, OK 1995.

	df	Forage	Grain	Straw	Forage	Grain	Straw	Grain + Straw
		------ yield (kg ha^{-1}) --------			---------- nitrogen uptake (kg ha^{-1}) -------			
Treatment	37	*	**	**	**	**	**	**
Error	69							
Contrasts:								
TAM W-101:								
45 preplant vs 45 split	1	ns	ns	ns	ns	ns	ns	ns
90 preplant vs 90 split	1	ns	ns	ns	ns	ns	ns	ns
180 preplant vs 180 split	1	ns	ns	ns	ns	ns	ns	ns
Longhorn:								
45 preplant vs 45 split	1	ns	ns	ns	ns	ns	ns	ns
90 preplant vs 90 split	1	ns	ns	**	ns	ns	*	*
180 preplant vs 180 split	1	**	**	**	**	*	ns	*
Overall N rates:								
TAM W-101 vs Longhorn	1	**	*	**	ns	ns	**	**

*, ** Significant at 0.05 and 0.01 probability levels, respectively. Preplant - N applied at preplant and disc incorporated; Split - 1/2 N applied at preplant and disc incorporated and 1/2 applied topdress at Feekes 4.

Table 9. Analysis of variance and single degree of freedom contrasts on nitrogen use efficiency components as affected by split application of nitrogen, Perkins, OK 1995.

	df	Protein %	N-use efficiency (Gw/Ns)	N-uptake efficiency (Nt/ Ns)	N-utilization efficiency (Gw/Nt)	Fraction of N translocated to grain (Ng/Nt)	Grain yield/ grain N (Gw/Ng)	N loss (kg ha^{-1}) (Na-Nh)
Treatment	37	**	**	**	**	**	**	ns
Error	70							
Contrast:								
TAM W-101:								
45 preplant vs 45 split	1	ns	ns	ns	ns	ns	ns	ns
90 preplant vs 90 split	1	ns	ns	ns	ns	ns	ns	ns
180 preplant vs 180 split	1	ns	ns	ns	ns	ns	ns	ns
Longhorn:								
45 preplant vs 45 split	1	ns	ns	ns	ns	ns	ns	ns
90 preplant vs 90 split	1	ns	ns	ns	ns	ns	ns	ns
180 preplant vs 180 split	1	ns	ns	ns	ns	ns	*	*

*, ** Significant at 0.05 and 0.01 probability levels, respectively. Preplant - N applied at preplant and disc incorporated; Split - 1/2 N applied at preplant and disc incorporated and 1/2 applied topdress at Feekes 4.

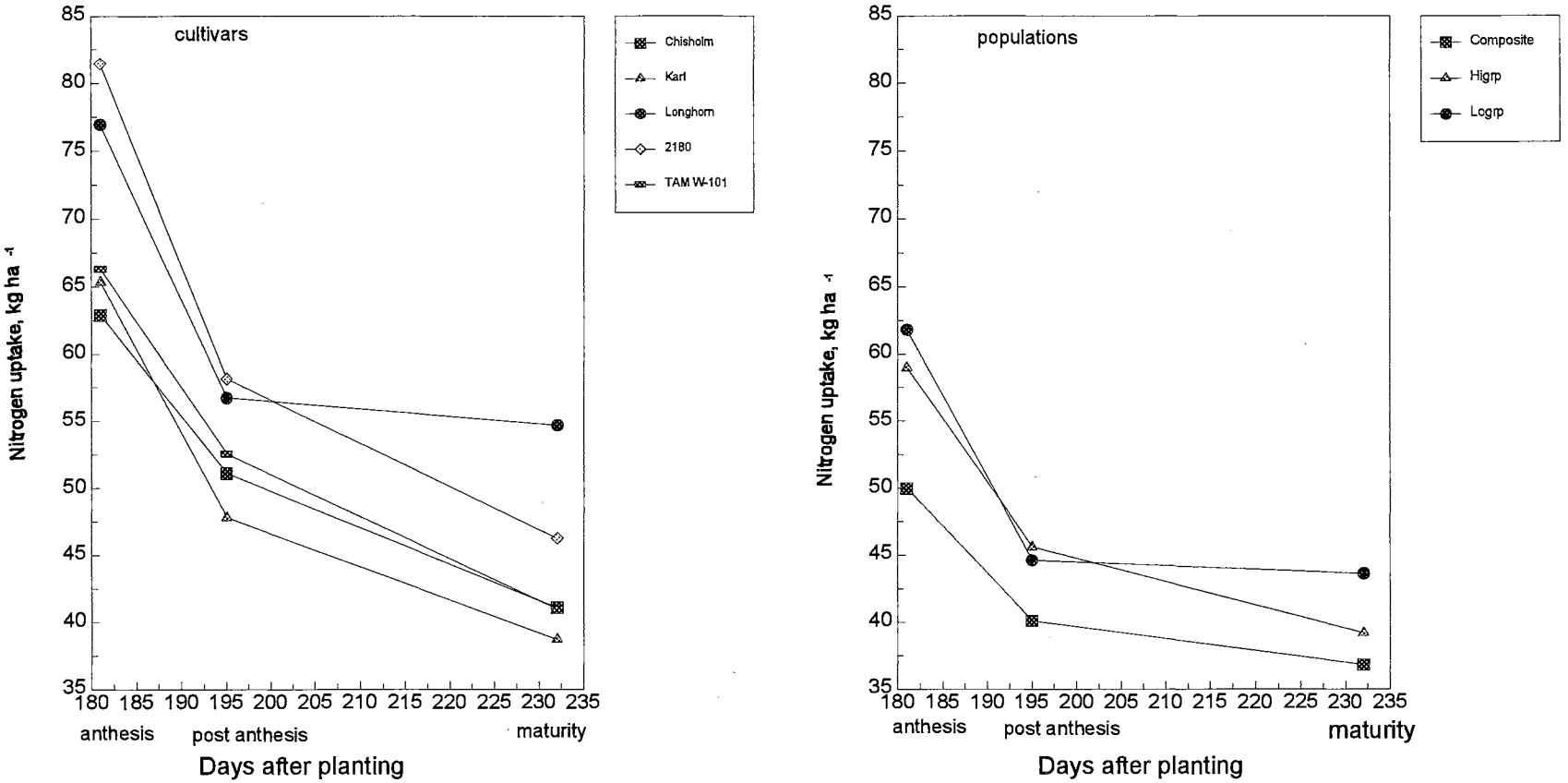

Figure 1. Change in total N uptake from anthesis to maturity in several winter wheat cultivars and populations, Perkins, OK 1995.

z(x,y) = 14.4893 + -20.1931y + 3.05327y^2 + -0.0146469x + 0.0104104xy + 8.86275e-007x^2
R2=0.77

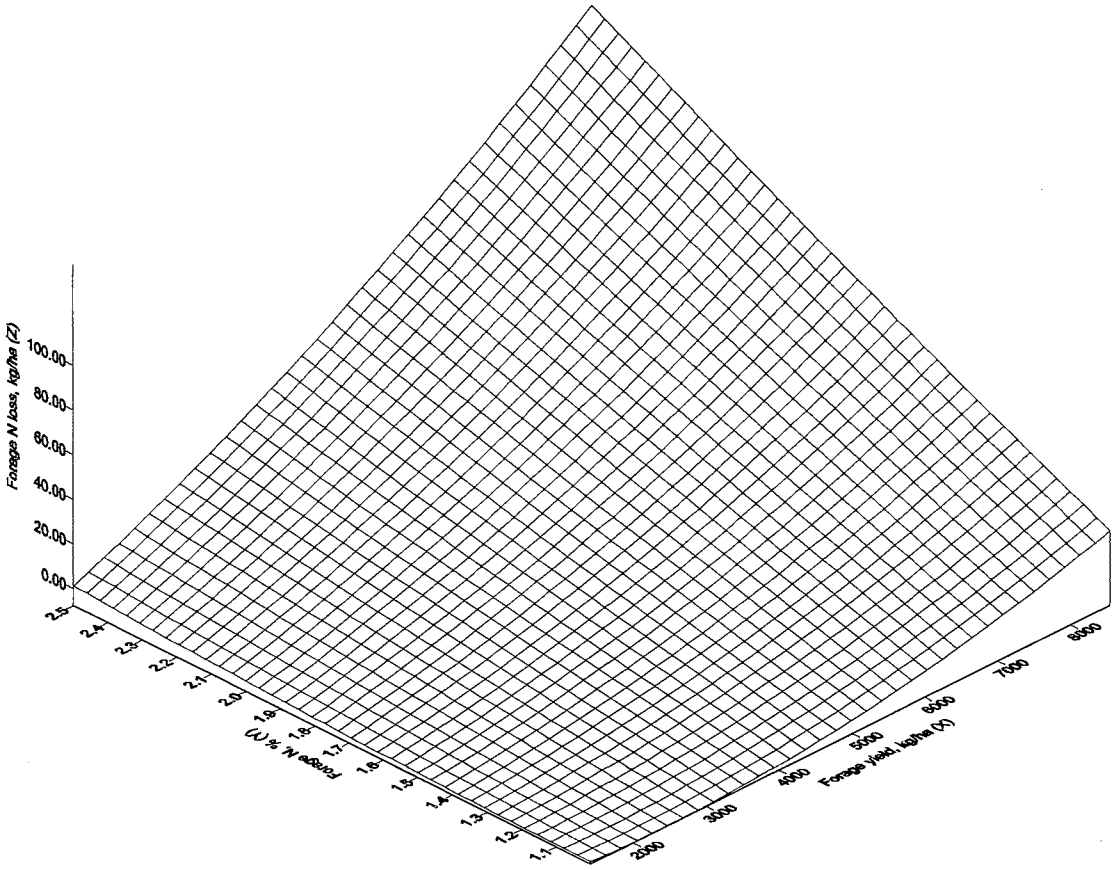

Figure 2. Response surface model of forage nitrogen loss versus forage yield and percent forage nitrogen, Stillwater, OK, 1994

z(x,y) = -33.588 + 15.6324y + -4.76397y^2 + -0.00173798x + 0.00876535xy + -6.57643e-007x^2
R2 = 0.73

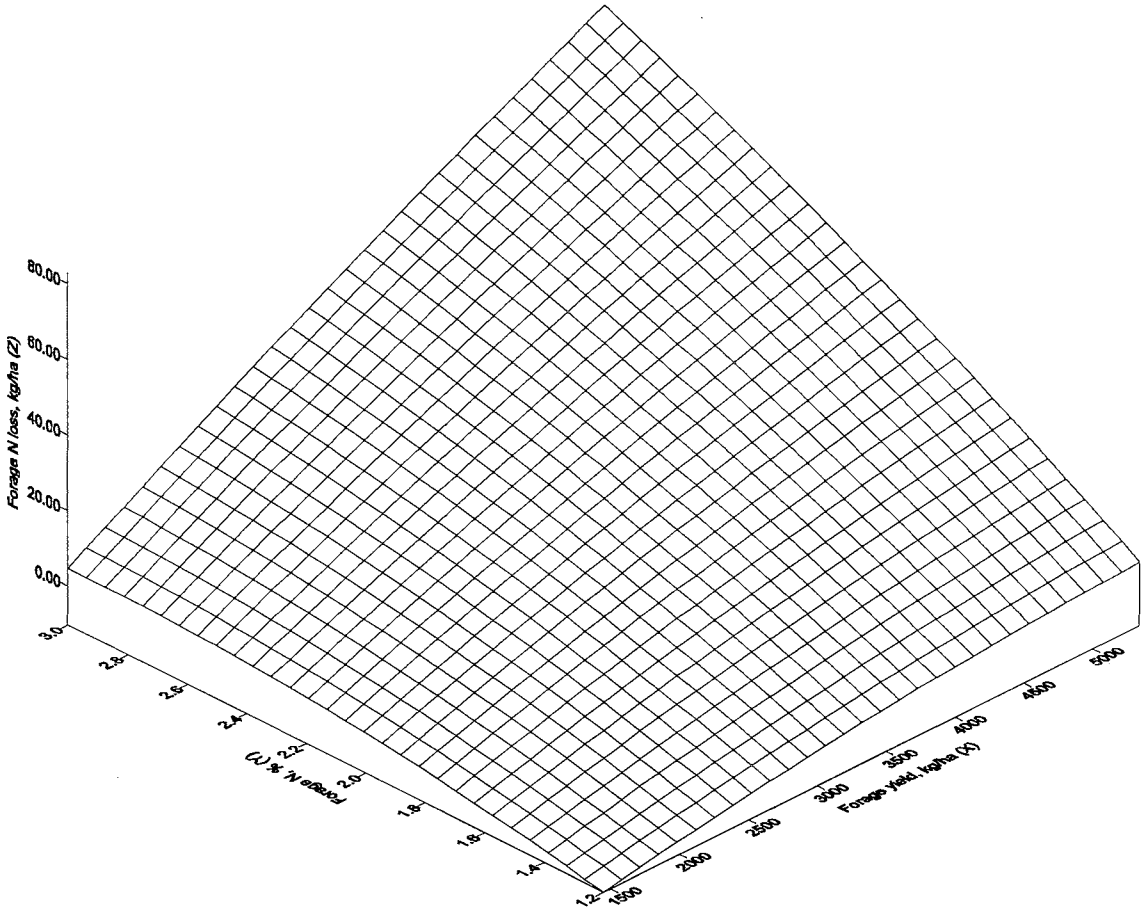

Figure 3. Response surface model of nitrogen loss versus forage yield and percent forage nitrogen, Perkins, OK 1995.

CHAPTER II

GLUTAMINE SYNTHETASE ACTIVITY IN WINTER WHEAT VARIETIES

ABSTRACT

Plant shoots may be a significant source of nitrogen (N) loss in crops resulting in lower nitrogen use efficiency. Nitrogen volatilization is thought to occur when ammonia (NH_3) is produced in excess and volatilized to the atmosphere through the stomata. Theoretically the enzyme glutamine synthetase (GS) should counteract NH_3 volatilization by assimilating the excess tissue NH_3 into nonvolatile amino acids. The objective of this project is to determine the relationship between GS activity and N use parameters. One field experiment was initiated in October 1994 at the Agronomy Research Station, Perkins, OK to measure GS activity, leaf ammonium (NH_4^+) and N use efficiency parameters in five winter wheat cultivars, and three experimental populations. Two N levels of 45 and 180 kg N ha^{-1} were utilized using urea ammonium nitrate (UAN, 28-0-0) as a N source. Leaves from the upper part of the wheat plant were analyzed for GS activity, NH_4^+ concentration and total protein. There were no significant entry

differences in GS activity, leaf NH_4^+ and N loss, but there were entry differences in N use efficiency and protein concentration at anthesis. Nitrogen use efficiency varied significantly with respect to N level but not with GS activity, protein concentration, NH_4^+ concentration and N loss. There was a positive correlation between GS activity and N loss (r^2 = 0.34). Plant ammonium at anthesis was negatively correlated (r^2 = -0.42) with final grain N at harvest, but was not correlated with N loss between anthesis and harvest. Leaf protein concentration was negatively correlated (r^2 = -0.26) to percent grain N at harvest. The positive correlation between GS activity and N loss suggests that GS activity at anthesis may serve as a marker for N loss between anthesis and harvest, however, further studies are required to verify this.

INTRODUCTION

The effectiveness with which N is used by wheat and other cereals has become increasingly important because of increased costs associated with the manufacture and distribution of N fertilizer. Nitrogen is an essential element for plant growth, plays a crucial role in crop production, and is usually the most costly input used to produce non-legume crops. Identification and development of cultivars that can absorb N more efficiently from the soil and effectively partition this N into the grain could potentially minimize loss of soil N and make more economic use of the absorbed N (Dhugga and Waines, 1989). Research on N use efficiency has been stimulated by the accumulation of evidence that

crop recovery of fertilizer N is only about 50 % of that applied. The major potential sources of N fertilizer loss include gaseous plant loss, volatilization, immobilization, denitrification and leaching.

Volatile NH_3 from plant shoots is the prevalent form of post-anthesis N loss (Harper *et al.*, 1987). Nitrogen loss from wheat plants as NH_3 has also been found during periods when there is adequate available soil N (Harper *et al.*, 1987) and during plant senescence (Harper *et al.*, 1987; Parton *et al.*, 1988). The former NH_3 losses could have been due to overloading of plant N as NH_4^+ whereas the latter could be due to plant senescence and inefficient redistribution of N within the plant. Under field conditions, Harper *et al.* (1987) found a total loss of about 15 kg ha^{-1} of N as NH_3 from a wheat crop. Plant N loss in wheat was estimated as the difference between total forage N at anthesis and total (grain + straw) N at harvest (Daigger et al., 1976). Nitrogen loss from the wheat plants increased as the amount of N applied was increased, with an N loss from anthesis to maturity ranging from 25 kg ha^{-1} where no N was applied to 80 kg ha^{-1} when 150 kg N ha^{-1} were applied.

Nitrogen remobilized from proteins in leaves and stems during senescence is a very important source for grain protein formation. This nitrogen must be reassimilated into transportable amino acids (glutamine and asparagine) involving the release of ammonia. In cereal grasses, 50-80 % of the N present in vegetative plant parts at anthesis was retranslocated to the head (Harper *et al.*, 1987). Van Sanford and Macknown (1987), working with soft red winter wheat, detected significant cultivar differences in remoblized N from the

flag leaf, peduncle, and lower culm. The proportion of N accumulated by the spike ranged among cultivars from 51 to 91 %t. They also found approximately 83 % of the total above ground N at physiological maturity was present in the plant at anthesis. Post-anthesis N uptake was associated with lower N utilization efficiency (spike wt/total plant N), higher grain N concentration and lower grain yields (Van Sanford and Macknown, 1987).

The key step in the conversion of ammonia (NH_3) to nonvolatile organic N is catalyzed by the enzyme GS (Joy, 1988). Glutamine synthetase has a high affinity for NH_3 and is recognized as the primary enzyme in the process of remobilization of N for transport in plants (Simpson and Dalling, 1981). Glutamate reacts with NH_4^+ to form glutamine, this reaction is catalyzed by GS. The amide N of glutamine is transferred to α–ketoglutarate to form glutamate which is readily utilized to form other amino acids. The combined action of GS and glutamate synthase has been termed the glutamate synthase cycle (Joy, 1988). Operation of the cycle both maintains the cycle and results in a net production of glutamate, which can be utilized as a source of amino N for the synthesis of proteins or for transport to the developing grain. The activities of GS and glutamate synthase exert partial control over the level of NH_4^+ in the leaf tissue. Nitrogen losses from plants appears to be closely associated with the level of ammonium in leaf tissue (Schjoerring *et al.* 1993b).

The rate and direction of plant NH_3 fluxes is a function of the gradient in NH_3 molar fractions between the substomatal cavities and the ambient

atmosphere. Emission of NH_3 will take place when the molar fraction of NH_3 in the substomatal cavities than in the atmosphere. The molar fraction of NH_3 in the substomatal cavities depends on temperature, pH and NH_4^+ concentration in the leaf apoplast. Schjoerring *et al.* (1993a), who studied the exchange of NH_3 between the atmosphere and the canopy of spring barley crops grown at three levels of N application, found that emission of NH_3 to atmosphere started around two weeks before anthesis, and peaked about or shortly after anthesis. Loss of NH_3 from the canopy increased with the N status of the canopy (Schjoerring *et al.*, 1993a).

In addition to the net flow of N through NH_3 during inorganic N assimilation, further massive cycling of N through NH_3 occurs during photorespiration in illuminated leaves of C_3 (and possibly C_4) plants. This flow can be as much as 10-fold greater than the net assimilation of inorganic N, which may also be occurring in the leaf (Wallsgrove *et al.*, 1983). Assimilation of this vast amount of NH_3 is through cytoplasmic or chloroplast GS (Keys *et al.*, 1978). The influence of N source-sink relationships on the balance between NH_4^+ releasing and assimilating processes is still not known. Since very low concentrations of ammonium are phytotoxic, emission of NH_3 through stomata (Schjoerring *et al.*, 1993b) may be a useful protective mechanism against accumulation of toxic levels of ammonium in leaf tissue, especially under conditions with limited sink capacity for N. Little is known about the magnitude of NH_3 losses from plants under field conditions and how plant-derived NH_3 emissions vary with environmental variables and with N status of the plants.

Schjoerring *et al.* (1993b) found declining GS and glutamate synthase activities with leaf age in different leaves of field-grown spring barley during the reproductive growth phase in two consecutive years. Similar findings were reported in wheat (Simpson and Dalling, 1981) and in rice (Kamachi *et al.*, 1991). The decline in enzyme activities was followed by an increase in soluble NH_4^+ and amides in the leaf tissue. Ammonia volatilization occurring under conditions with declining GS and glutamate synthase activities and increasing tissue concentrations of NH_4^+ may be useful mechanisms to protect the plant from toxic accumulation of tissue NH_4^+ and NH_3.

Assimilation of NH_3 into organic forms is a vital and very active process in plants. In spite of the extensive knowledge on primary N assimilation, it is not fully understood how GS activity is related with N use efficiency in crops like wheat at various N fertilization rates. The objective of this study was to determine the inherent variability of GS activity and its relationship to N use efficiency and estimates of plant N loss in several winter wheat varieties and experimental lines at two N levels.

MATERIALS AND METHODS

One field experiment was initiated in October 1994 at the Agronomy Research Station, Perkins, OK to measure GS activity, leaf ammonium and N use efficiency at two N levels. A total of 5 wheat cultivars (Karl, 2180, TAM W-101, Chisholm and Longhorn) and 3 experimental populations were evaluated.

The experimental populations consisted of an unselected hard winter wheat population (Control Composite) and the same population subjected to two cycles of selection for either high (Higrp) or low (Logrp) yield potential under sub-optimal N application. All the cultivars and experimental populations were evaluated at preplant N rates of 45 and 180 kg N ha^{-1}. Urea ammonium nitrate (UAN, 28-0-0) was used as the N source, all of which was broadcast and incorporated at planting. A complete factorial arrangement of treatments was used (N rate x genotype) in a randomized complete block experimental design with three replications. The soil type at Perkins was a Teller sandy loam (fine-loamy, mixed, thermic Udic Argiustoll). Each plot consisted of five rows spaced 0.225 m apart and 15.2 m in length. Dry matter forage yields were obtained at anthesis on April 24th and May 8th (anthesis and fourteen days post-anthesis), from an area 0.45 x 3 m. Grain and straw yield were determined by harvesting a 1.125 x 9 m area on July 14th m using a self propelled combine. Sufficient area was available in each plot to accommodate both forage harvests (anthesis and fourteen days post-anthesis) and grain yield in separate areas of each plot. Forage harvests were obtained by hand clipping all plants 2 cm above ground. Subsamples from each respective harvest were collected for moisture and total N analysis. All forage and grain samples were ground in a large Wiley mill and later in an automated grinding unit to obtain finely ground forage, grain and straw samples. Total N was determined on forage, grain and straw samples using a Carlo-Erba NA 1500 dry combustion analyzer (Schepers *et al.*, 1989). Nitrogen

use efficiency for grain yield was partitioned into various components according to an expanded model of Moll *et al.*, 1982. Plant N loss was estimated as the difference between total forage N at anthesis and the total (grain + straw) N at harvest.

In addition to whole plot forage samples, leaves from the upper part of the wheat plant were taken at anthesis (April 24th, 1995) and immediately put on ice. These leaves were then stored in a cold room at 4°C and analysis for GS activity, NH_4^+ and protein determination accomplished within three days. Preliminary work showed storing leaves at 4°C for upto six days had no effect on Gs activity. Glutamine synthetase activity was performed according to Groat and Vance (1981). Five grams of leaves were homogenized for 30 seconds at 4 °C with 25 ml extraction buffer at pH 6.8 consisting of 100 mM MES-NaOH, 100 mM sucrose, 2 % B mercaptoethanol, 15 % ethylene glycol. Ten ml of homogenates were centrifuged at 10,000 rpm for 20 minutes and PMSF was added to the supernatant to 0.1 mM. The resulting fraction was assayed for GS activity (nmol glutamine g^{-1} fresh weight min^{-1}).

Glutamine synthetase assays were conducted by mixing 100 mM NH_4^+, 50 mM ATP, 55 mM $MgSO_4$, 100 mM [UL-^{14}C]glutamate, 0.5M Tricine and water at pH 7. Fifty µl extract was added to 200 µl complete assay mixture giving a final volume of 250 µl. The reaction was terminated after 30 minutes by adding 1 ml of ice-cold water. One ml columns of Dowex-1 were used to separate [^{14}C]glutamine from unreacted [^{14}C]glutamate. Glutamine synthetase

activity was determined by measuring the amount of radioactivity as glutamine. Four ml of ice water was added to the columns and the eluant was collected. Product formed in the eluant was measured for radioactivity using liquid scintillation spectrometry. Assay controls consisted of reaction mixtures lacking enzyme.

Ammonium ($\mu g\ g^{-1}$ fresh weight) determination in leaf tissue was performed according to Martin *et al.* (1983). One ml extract was added to a flask with a center well containing 0.5 ml of 0.1 N HCl. Two ml of 0.5 M sodium tetraborate (pH 10) were injected into the flask, being careful to by-pass the center well and the flask was immediately stoppered to avoid loss of ammonia. Incubation was for 40 hours after which a total N assay on contents of the center well was performed. A 0.25 ml sample from the center well was taken and 2.5 ml of nitroprusside/phenol mixture, 2.5 ml $NaOH/Na_2HPO_4/NaOCl$ was added to the sample. The sample contents were thoroughly mixed using electric vortex mixer and incubated in a water bath at 37°C for 30 minutes. Absorbance was read with a spectrophotometer at 625 nm. Blank and standard assays with known concentrations of NH_4^+ as an internal standard and extract were performed. Protein assays were performed to measure the total protein concentration (mg g^{-1} fresh weight.) using Bio-Rad Protein Assay according to manufactures directions (Bradford, 1976). Data analysis was performed using SAS (SAS Institute Inc., 1988), and means comparisons was done using Student-Newman-Kuels' (S-N-K) test at significance level of 5%.

RESULTS AND DISCUSSION

Analysis of variance and single degree of freedom contrasts for N rate, entry and their interactions on GS activity, protein, NH_4^+, and N use efficiency variables are provided in Table 1. Significant differences among entries were apparent for leaf protein concentration, but not for GS activity and leaf NH_4^+ levels at anthesis. Leaf protein concentration ranged from 3.60 to 4.69 mg g^{-1} fresh weight for Logrp and Control Composite, respectively (Table 2). No significant differences existed for GS activity, leaf protein concentration or leaf NH_4^+ concentration at the N rates of 45 and 180 kg ha^{-1}. Logrp and Chisholm had the lowest and highest GS specific activity, respectively, ranging from 680 to 847 nmol glutamine g^{-1} fresh weight min^{-1}.

GS activities in eight wheat entries measured at anthesis were positively correlated with N loss (r^2 = 0.34) between anthesis and harvest (Table 3). Nitrogen loss after anthesis is thought to be due to volatilization as NH_3 gas (Harper *et al.*, 1987). The rate of volatilization depends on the concentration differential of NH_3 between the substomatal cavity and the atmosphere (Schjoerring *et al.*, 1993a). Increased protein degradation during and after anthesis should have resulted in increased NH_3 production and higher N loss through NH_3 volatilization. Glutamine synthetase activity should have counteracted the potential loss by assimilating more NH_3 into non-volatile amino acids. Our results suggest the opposite, that high GS activities at anthesis were

positively correlated with high N losses between anthesis and harvest. The physiological or biochemical reason for this apparent correlation is unclear at this time and requires further verification.

Ammonium and protein concentration in leaf tissue of the eight wheat entries at anthesis was negatively correlated (r^2 = -0.43, r^2 = -0.26) with final grain N at harvest, but was not correlated with N loss between anthesis and harvest (Table 3). Varieties with high leaf NH_4^+ may show higher rates of N loss due to NH_3 volatilization, and therefore less N available for transport to the developing grain. The lack of correlation between NH_4^+ concentration in leaves at anthesis and N loss between anthesis and harvest does not support this explanation. However the high variability in N loss measurements (CV of 66 %) makes it difficult to have complete confidence in any interpretation of the N loss data without further verification. The significant negative correlation between protein content in the leaves at anthesis and grain N (r^2 = -0.26) suggests that varieties with high leaf protein may exhibit lower rates of remobilization of protein N and less N available for transport to the grain.

One of the objectives of this study was to identify potential markers for enhanced N use efficiency and reduced N losses to the environment. The positive correlation between GS activity and N loss suggests that GS activity at anthesis may serve as a marker for N loss between anthesis and harvest. However, our results disagree with what one would expect based upon our knowledge of biochemical and physiological mechanisms governing N assimilation and NH_3 volatilization. Moreover, there was no correlation between

GS activity and N use efficiency suggesting that GS may not serve as a marker for improved grain N use efficiency in wheat. Clearly, further study is warranted to confirm these results. Furthermore, GS, NH_4^+ concentration and leaf protein concentration measurements represent single time point measurements. These values may not adequately represent what happens in the plant from anthesis to harvest. More sampling under controlled environmental conditions needs to be performed to more adequately monitor the changes in GS activity, NH_4^+ concentration and protein concentration over time, and how these variables are statistically associated with N use efficiency parameters.

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Table 1. Factorial arrangement of treatment analysis of variance and comparisons for GS activity, protein, ammonium and N use efficiency variables, Perkins.

	df	GS activity	Protein (mg g^{-1} fwt.)	Ammonium (μg g^{-1} fwt)	GN (%)	N Loss (kg ha^{-1}) (Na-Nh)	NUSE (Gw/Ns)
Replication	2	NS	NS	**	**	NS	NS
Nrate	1	NS	NS	NS	**	NS	**
Entry	7	NS	**	NS	**	NS	**
Nrate*Entry	7	NS	NS	NS	NS	NS	*
Error	27	20937	0.16	2.53	0.05	250	8.7
R^2		0.29	0.64	0.67	0.82	0.44	0.92
C.V. %		19	10	28	8	66	23
Contrasts:							
Higrp vs Logrp	1	NS	NS	NS	NS	NS	NS
Higrp vs Composite	1	NS	**	NS	NS	NS	NS
Logrp vs Composite	1	NS	**	NS	NS	NS	NS
Higrp & Logrp vs Composite	1	NS	**	NS	NS	NS	NS

*, ** Significant at 0.05 and 0.01 probability levels, respectively; GS = glutamine synthetase; NUSE = nitrogen use efficiency; GN = % grain nitrogen; Na = N accumulated in plant at anthesis; Nh = N accumulated in plant at harvest; Gw = grain weight; Ns = N supply; fwt. = fresh weight; C.V. = coefficient of variation.

Table 2. Effect of variety on GS activity, ammonium, protein and components of N use efficiency (averaged over N rates), Perkins.

Entry	GS activity	Protein (mg g^{-1} fwt)	Ammonium ($\mu g\ g^{-1}$ fwt.)	GN (%)	N Loss (kg ha^{-1}) (Na-Nh)	NUSE (Gw/Ns)
Chisholm	847a	3.85bc	5.94a	3.0ab	25.7a	13ab
Composite	825a	4.69a	6.66a	3.1ab	16.6a	9b
Higrp	763a	3.65c	6.12a	3.2a	17.1a	11ab
Karl	777a	4.05abc	4.86a	3.1ab	32.5a	14ab
Logrp	680a	3.60c	5.76a	3.1ab	17.8a	11ab
2180	768a	4.20abc	5.76a	3.0ab	36.0a	17a
TAM W-101	801a	4.41ab	5.76a	2.6c	30.9a	13ab
Longhorn	746a	4.19abc	4.50a	2.7bc	27.7a	16a

Means followed by the same letter(s) are not significantly different at the 0.05 probability level. S-N-K used for mean comparisons. GS = glutamine synthetase; NUSE = nitrogen use efficiency; GN = % grain nitrogen; Na = N accumulated in plant at anthesis; Nh = N accumulated in plant at harvest; Gw = grain weight; Ns = N supply; fwt = fresh weight

Table 3. Correlation analysis of GS activity, ammonium and protein with N use efficiency variables, Perkins.

	AMMONIUM	PROTEIN	GN	NUSE	FNLOSS
GS ACTIVITY	-0.002	0.21	0.07	-0.13	0.34
	0.99	0.16	0.66	0.41	0.02
	45	45	45	45	45
AMMONIUM		0.14	-0.43	-0.04	-0.16
		0.36	<0.01	0.78	0.28
		45	45	45	45
PROTEIN			-0.26	0.09	0.16
			0.09	0.54	0.30
			45	45	45
GN				-0.32	0.19
				0.03	0.22
				45	45
NUSE					-0.21
					0.17
					45

GS = glutamine synthetase; NUSE = nitrogen use efficiency; GN = % grain nitrogen; FNLOSS = nitrogen lost between anthesis and harvesting.

CHAPTER III

EFFECT OF NITROGEN FERTILIZATION AND CATION REMOVAL ON SOIL pH

ABSTRACT

Crop yields are limited by low soil pH in areas subjected to continuous nitrogen (N) fertilization, and forage and grain removal. This study was conducted to evaluate the effect of continuous N fertilization and cation removal in forage on soil pH and exchangeable cations. One greenhouse pot experiment was initiated using a Teller sandy loam soil (fine-loamy, mixed, thermic Udic Argiustoll) and a Kirkland silt loam soil (fine-mixed, thermic, Udertic Paleustoll) from Perkins and Stillwater, respectively. Treatments included four N rates and five forage management schemes in a complete factorial arrangement. A randomized complete block experimental design was used with three replications. Six-liter pots were filled with 3,000 g of soil. Sorghum-sudan hybrid (*Sorghum sudanense L.*) and winter wheat (*Triticum aestivum L.*) 2180 were grown during hot and cool seasons, respectively. Soil pH values were

significantly decreased as a result of N fertilization for both Perkins and Stillwater soils. At the highest N rate soil pH decreased by 1.55 and 1.27 units in Perkins and Stillwater soils, respectively. Electrical conductivity (EC) increased with N application and was highest in forage management treatments where no crops were grown. Soil pH was lowest in the forage management treatment where no crops were grown. Where crops were grown, uptake of anions including NO_3^- may have resulted in release of OH^- to the soil solution to neutralize the H^+ produced during nitrification. Similarly, uptake of cations is expected to release H^+ to the soil solution. Where no crops were grown, there was no OH^- generated to neutralize the H^+ nor H^+ generated to increase it. Soil pH decreased with continuous cation removal by forage growth, with r^2 of 0.357 and 0.559 for Perkins and Stillwater soils, respectively. Perkins soil is coarse-textured with low buffering capacity whereas Stillwater is fine-textured with medium buffering capacity. This explains why the Perkins soil had greater decrease in soil pH than the Stillwater soil with increased N fertilization and continuous cation removal. Growing crops lowers the solution EC due to uptake of cations and anions which contribute to EC. The large values of EC with high rates of applied N indicate salinity could also have contributed to reduced plant growth at high N rates. Calculations show that EC of approximately 4 dS m^{-1} in the saturated soil condition may have existed for Perkins and Stillwater soils. Such values could have contributed to poor plant growth.

INTRODUCTION

Soil pH is an important soil property because it strongly influences soil chemical properties, availability of plant nutrients, and can have a significant effect on crop production. However, soils with the same pH do not necessarily have the same properties or limitation for plant growth, nor can the same response to pH change be expected, depending on other soil chemical and physical characteristics. Crops vary in their tolerance and ability to grow in soil at very high and very low pH (Allen and Johnson, 1992). Well-drained, productive soils under good management will slowly become acidic because acidity is a natural result of high crop production (Sanchez, 1976; Allen and Johnson, 1992). This could be slowed down by avoiding straw removal at harvest. Straw contains relatively higher amount of bases compared to grains. As crops remove bases from soil solution, bases adsorbed on soil solids move to the soil solution and replenish the supply. Because of this relationship and the large reserve of bases from soil solids, soil pH does not change much from month to month or even year to year (Allen and Johnson, 1992). Time required for neutral and basic soils to become acid will also depend on amount of rainfall, soil texture, reserve of basic minerals (Allen and Johnson, 1992), and the use (frequency and amounts) of ammoniacal fertilizers (Bohn *et al.,* 1985). Extremely acid soils may not be productive because of the presence of increased amounts of toxic elements such as aluminum (Al) and manganese

(Mn) (Sanchez, 1976; Conto, 1982; Bohn *et al.,* 1985; Allen and Johnson, 1992), and deficiency of calcium (Ca) or magnesium (Mg) (Sanchez, 1976), resulting in poor crop growth. Toxic levels of Al restrict root development and, as a result, nutrient and water uptake is also limited. Soil pH also affects the availability of nutrients for plants. Phosphorus (P) and molybdenum (Mo) availability are greater at near neutrality, but boron (B), iron (Fe), zinc, and Mn availability increase at low pH (Bohn *et al.,* 1985). Microbiological activities are also influenced by soil pH, which affects symbiotic and free living nitrogen (N) fixation, organic P mineralization, and the population of fungi- and bacteria-producing plant diseases. Because yield and plant productivity are functions of these and other factors, the importance of soil pH on plant productivity can be easily visualized.

The basic cations in plant ash cause dramatic increases in exchangeable Ca, Mg, potassium (K), and sodium (Na) after crop burning (Sanchez, 1976). This is followed by a gradual decrease in exchangeable bases during the cropping period due to leaching and crop uptake. The magnitude of these changes varies with soil and ash composition. Soil pH increases after burning because of the incorporation of basic cations (in the form of oxides and hydroxides), and gradually decreases with dilution in the soil by cultivation. In acid soils these changes are beneficial because they decrease or precipitate the exchangeable Al. In high-base-status soils the ash may raise the pH to 7 or 8 possibly causing detrimental effects such as Fe and P deficiency. In all cases

the soil tests for available P and K may increase after burning because of the contribution of the ash.

Sources of Soil Acidity

Nitrogen Fertilization

Continued crop fertilization with ammonia or ammonium fertilizer can lead to acidic soil conditions. Nitrification of ammoniacal N fertilizers results in the production of hydrogen ions that contribute to increased soil acidity (Sanchez, 1976; Westerman, 1981; Bohn *et al.*, 1985; Tisdale *et al.*, 1985). All fertilizers containing N in the form of ammonium will, by natural microbial processes, nitrify utilizing specific soil bacteria.

$2NH^+_4 + 3O_2$ Nitrosomonas spp. ===> $2NO^-_2 + 4H^+ + 2H_2O$ (1)

$2NO^-_2 + O_2$ Nitrobacter spp. ===> $2NO^-_3$ (2)

$2NH^+_4 + 4O_2$ ==> $2NO^-_3 + 4H^+ + 2H_2O$ (overall reaction) (3)

In alkaline soils, acidity produced from nitrification would be beneficial. However, in soils that are slightly acid (pH 5.5-6.5) the potential for reduction in yields exists with continued use of acid-forming fertilizers unless corrective lime applications are made (Westerman, 1981).

Crop Cation Removal

Basic cation removal in forage, grain and straw is also a contributing factor to soil acidity (Westerman, 1981; Tisdale *et al.*, 1985). Removal of straw

depletes the bases in soil faster than removal of N which enhances the acidification effect of fertilizer N. Therefore, continuous application of ammoniacal fertilizers and removal of straw may often result in decreased soil pH.

Decomposition

Organic residue decomposition in soil also contributes to increased soil acidity. Carbon dioxide is released from organic residues during decomposition and combines with water to form carbonic acid (Bohn *et al.*, 1985). Carbonic acid dissociates into hydrogen ions (H^+) and bicarbonate ions (HCO^-_3), resulting in another source of H^+ for increasing soil acidity in a cropping system. Root activity and metabolism may also serve as a source of CO_2 and acid secretions. Decomposition of organic waste produces organic acids which increase soil acidity (Bohn *et al.*, 1985; Tisdale *et al.*, 1985) and also tie up Al.

Leaching

When rainfall exceeds evapotranspiration, leaching exchangeable bases out of the effective root zone can take place. They are replaced first by H^+ and subsequently by Al ions making the leached soil surface slightly to moderately acid (Westerman, 1981; Bohn *et al.*, 1985; Tisdale *et al.*, 1985). This is not a major factor in fine-textured soils with high buffering capacity and cation

exchange capacity (CEC). However, in sandy soils with low buffering capacity and CEC, leaching is a more serious problem when rainfall is high.

Problems of Soil Acidity.

Soil acidity may be beneficial or detrimental to plant growth. Legumes require a neutral or alkaline soil pH for best growth (Fraps and Fudge, 1932; Tisdale *et al.*, 1993), therefore, soil acidity would greatly affect their performance. Soil acidity has beneficial effects where certain plant diseases may be a problem, such as potato scab and cotton root rot, which are not present in acid soils.

Soil pH *per se* has no direct effect on plant growth, except at pH values below 4.2, where the H^+ concentration may stop or even reverse cation uptake by roots (Sanchez, 1976). However, soil pH is a good index of the soil chemical environment; it is best indicator of Al toxicity. Soil solution concentrations of Al above 1 ppm often cause direct crop yield reduction from root injury. As a result, root development is restricted, and the roots become thicker and stubby and show dead spots (Sanchez, 1976). Aluminum toxicity decreases root growth and the translocation of Ca and P to tops. Aluminum toxicity can be corrected by liming to pH 5.5-6.0, to precipitate the exchangeable Al as Al hydroxide (Sanchez, 1976; Bohn *et al.*, 1985). Liming may also increase P and Mo availability (Bohn *et al.*, 1985).

In general, the availability of micronutrients and toxic metal cations increases with increasing soil acidity. Those present as anions (Mo, and sometimes B) differ in that their availability generally increases with increasing soil pH (Bohn *et al.*, 1985). Occasionally, the harmful effect of soil acidity on leguminous plants seems to be caused by Mo deficiency rather than Al toxicity. Mo is required for N fixation by legumes.

These factors have not been quantified although continuous use of N fertilizers, crop cation removal and leaching are thought to be the major causes of acidity. This study was initiated to a) evaluate the effect of continuous N fertilization on soil pH, and b) to determine the relationship between continuous cation removal in forage with soil pH and exchangeable cations.

MATERIALS AND METHODS

One greenhouse experiment was initiated in June 1993 using a Teller sandy loam soil (fine-loamy, mixed, thermic Udic Argiustoll) (low buffering capacity) and a Kirkland silt loam soil (fine-mixed, thermic, Udertic Paleustoll) (medium buffer capacity) from Perkins and Stillwater, respectively. Bulk surface soil (0-15 cm) was collected from each site, allowed to dry, ground to pass a 20 mesh screen, and 180 kg thoroughly mixed with a cement mixer to make it relatively homogenous. Initial chemical characteristics for each soil are reported in Table 1. Treatments included four N rates and five forage management

schemes in a complete factorial arrangement (Table 2). A randomized complete block experimental design was used with three replications.

Six-liter pots were filled with 3,000 g of soil. Sorghum-sudan hybrid (*Sorghum sudanense L.*) and winter wheat (*Triticum aestivum L.*) 2180 were grown during hot and cool seasons, respectively. Ten seeds of either crop were planted and thinned to six seedlings per pot five days after germination. Pots were watered using distilled water to avoid addition of any minerals (bases). A plate was placed at the bottom of the pots to trap any bases that might have leached through the soil. Leached water was later added back to the pots. Nitrogen was applied at rates of 0, 160, 320, and 480 kg N ha^{-1}. Half of the N was applied at planting and the other half five weeks later.

Wheat and sudan forage were harvested by cutting plants at the base when the wheat reached boot stage and the sudan reached the 8 leaf stage. Forage was oven dried at 70°C for 24 hours and weighed to determine dry matter yield. After weighing, forage samples were ground to pass a 100 mesh screen. Forage from selected treatments were ashed at 500°C for 4 hours (Jones and Case, 1990). Following harvest, soils were thoroughly mixed and sampled before ground and ashed forage was added to pots, in respective treatments, and thoroughly mixed before planting the next cycle. About 10 and 0.5 g of dried soil and ground forage, respectively, were taken for analysis at the end of every cycle. Soil samples were analyzed for pH (1:1 soil/water (w/v)); CEC (Polemio and Rhoades, 1977); exchangeable cations and base saturation.

Analysis of Ca, Mg, K, and Na in forage and soil was accomplished using an atomic absorption spectrophotometer. Soil and forage total N and soil organic C were analyzed using a Carlo-Erba NA 1500 dry combustion analyzer. Planned analyses of soils and harvested plant material between cycles were not implemented because forage yield was very low due to poor plant growth. Electrical conductivity (1:5 soil/water [w/v] extract) was also determined at the end of the study (Rhoades, 1982).

Analysis of variance of data collected was performed by individual cycle. Single degree of freedom non-orthogonal contrasts were used to detect statistical differences between treatments.

RESULTS AND DISCUSSION

Analysis of variance and single degree of freedom contrasts for N rate on pH and EC for various cycles for the two soils are shown in Table 3. Cycle 3 data represents conditions after terminating the experiment. Cumulative N applied over 3 cycles was 320, 880, 1,440 and 2,000 kg ha^{-1}. Soil pH and EC response to applied N was highly significant. A linear decrease in pH and increase in EC, with increasing applied N was found (Table 3, Figures 1 and 2). Cycle 2 had consistently lower soil pH than cycles 1 and 3 except for the highest N rate for the Stillwater soil where it was higher than for cycle 3. Cycles 1 and 2 for the Perkins soil had quadratic responses in pH with increasing applied N

(Table 3 and Figure 1). At the end of cycle 3 soil pH had decreased from 6.47 to 5.84 (0.63) for Perkins and from 5.63 to 5.26 (0.37) for Stillwater for the lowest N rate (Tables 1 and 4). At the highest N rate, soil pH decreased from 6.47 to 4.92 (1.55) for Perkins and from 5.63 to 4.36 (1.27) for the Stillwater soil. The observed decreases in soil pH with increased ammoniacal N fertilizer in this study was probably due to the production of H^+ that contributed to increased soil acidity (Sanchez, 1976; Westerman, 1982; Bohn *et al.*, 1985; Tisdale *et al.*, 1985). The Perkins soil has a lower buffering capacity (CEC is about half that of Stillwater soil), as compared to Stillwater soil which is fine-textured. Increased applied N increased EC for both soils (Table 4 and Figure 2). However, the Stillwater soil had higher EC at all N rates. Unused inorganic N ions (NH_4^+ and NO_3^-) increased EC as applied N increased. Since the Stillwater soil had higher CEC and exchangeable cations than the Perkins soil (Table 1), this could be the reason why the Stillwater soil had higher EC.

Analysis of variance for forage management methods on pH and EC for various cycles for the two soils are shown in Tables 3 and 5, and Figures 3 and 4. Soil pH and EC response to forage management methods were highly significant. Forage management treatments where no crops were grown (NC and NA) had relatively higher EC and lower pH as compared to where a crop was grown (RR, AA and GA) in both soils (Table 5 and Figure 3). Cropping reduced EC due to utilization of cations and anions which contribute to EC. Where crops were grown, uptake of anions including NO_3^- may have resulted in

release of OH^- to soil solution to neutralize the H^+ produced during nitrification and hence the higher the soil pH. Similarly, uptake of cations is expected to release H^+ to the soil solution. Probably where crops were not grown there was a salt effect that enhanced the cation replacement of exchangeable H^+ resulting in a lower pH.

Except for pH during cycle 1 of the Stillwater soil, there was an interaction between N rate and forage management methods for pH and EC (Table 3, and Figures 5 and 6). Though EC for all forage management and for both soils increased with N applied, the increase was higher where no crops were grown (Figure 6).

Poor plant growth experienced in this experiment could have been caused by low soil pH and salinity, among others factors. When soil pH is <5.0 to 5.5, Al and Mn toxicity are probably the most important growth limiting factors. Excess Al interferes with cell division in plant roots; fixes P in less available forms in soils; decreases root respiration and interferes with uptake, transport, and use of nutrients and water by plants (Tisdale *et al.*, 1993). At pH 4.5 or less, H^+ damages root membranes and hence limits water and nutrient uptake by plants. In this experiment, soil pH values lower than 4.5 and 5.0 were observed in Stillwater and Perkins soils, respectively, and this may have contributed to poor plant growth. Soil pH decreased linearly and was correlated with cumulative N applied in both soils with r^2 of 0.431 for Perkins and 0.659 for Stillwater (Figure 7). The linear response of EC with applied N indicates salinity could also have

contributed to reduced plant growth. Calculations show that EC of 4 dS m^{-1} for a saturated soil solution would correspond with 0.25 and 0.27 dS m^{-1} (osmotic pressure (π) $\cong$ 1.56) in a 1:5 soil:water suspension for Perkins and Stillwater, respectively. Such values were observed at the termination of the experiment in both soils, and this could have contributed to poor plant growth (salinity tolerance of wheat and sorghum is moderate). No leaching of soluble salts was allowed as happens under field conditions. Increased salinity results in decreased osmotic potential and this could have reduced water and nutrient uptake by the plants.

Soil pH decreased with cumulative forage removal (Figure 8) and this also could have contributed to poor plant growth. As for cumulative N, soil pH associated with forage removal was lower for Perkins than Stillwater with r^2 for simple correlation of 0.357 and 0.559, respectively. These results suggest continuous cation removal in forage decreases soil pH.

CONCLUSIONS

Soil pH values were significantly decreased as a result of N fertilization during the 3 cycle experiment for both Perkins and Stillwater soils. Soil pH decreased with increasing N applied. At the high N rate soil pH decreased by 1.55 and 1.27 units in Perkins and Stillwater soils, respectively. The Perkins soil was coarse-textured with a low buffering capacity whereas the Stillwater soil was

fine-textured with medium buffering capacity. This explains why soil pH decreased more in the Perkins soil compared to Stillwater.

Electrical conductivity increased with N application and was highest in forage management treatments where no crops were grown. Growing crops lowered the EC due to uptake of cations and anions which contribute to EC. The large values of EC with high rates of applied N indicates salinity could also have contributed to reduced plant growth at high N rates. Calculations show that an EC near 4 dS m^{-1} in the saturated soil condition may have been approached for Perkins and Stillwater soils in which plants were grown. Such values could have contributed to poor plant growth. All salts which could have leached, as happens in field conditions, were added back and this could have resulted in increased salt and hence poor plant growth.

Soil pH was lower where no crops were grown. Where crops were grown, uptake of anions resulted in release of OH^- to soil solution which neutralizes the H^+ produced during nitrification. When no crops were grown, there was no OH^- generated to neutralize the H^+.

Soil pH decreased with continuous cation removal by forage growth, with r^2 of 0.357 and 0.559 for Perkins and Stillwater soils, respectively. Due to poor plant growth, we were not able to learn how continuous cation removal in forage affects exchangeable cations in these soils. Several growth cycles are required to achieve this and if problems encountered in this work are resolved, it would be exciting to investigate how cation removal in forage affects exchangeable cations.

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Table 1. Initial soil test characteristics and classification of soils used.

Characteristics	Perkins soil (Teller sandy loam)	Stillwater soil (Kirkland silt loam)
Classification	fine-loamy, mixed, thermic, Udic Argiustoll	fine-mixed, thermic, Udertic Paleustoll
pH	6.47	5.63
CEC ($cmol_c$/kg soil)	14. 69	24.70
Phosphorus (mg kg^{-1})	27.72	18.66
Exchangeable cations:		
Ca (mg kg^{-1})	205.5	523.5
Mg (mg kg^{-1})	181	334.5
K (mg kg^{-1})	181.5	199.5
Na (mg kg^{-1})	94.5	57
Base saturation (%)	106.1	95.8
Total nitrogen (g kg^{-1})	0.6	0.5
Organic carbon (g kg^{-1})	9.2	8.2

Table 2. Nitrogen rate and forage management factorial treatment combinations employed for Perkins and Stillwater soils.

Treatment	Nitrogen rate, kg ha^{-1}	Forage management
1	0	RR (forage removed)
2	0	GA (ground forage added)
3	0	AA (forage ashed and added back)
4	0	NC (No crop grown or forage added)
5	0	NA (No crop grown, ground forage from treatment 1 added back)
6	160	RR (forage removed)
7	160	GA (ground forage added)
8	160	AA (forage ashed and added back)
9	160	NC (No crop grown or forage added)
10	160	NA (No crop grown, ground forage from treatment 6 added back)
11	320	RR (forage removed)
12	320	GA (ground forage added)
13	320	AA (forage ashed and added back)
14	320	NC (no crop grown or forage added)
15	320	NA (No crop grown, ground forage from treatment #11 added)
16	480	RR (forage removed)
17	480	GA (ground forage added)
18	480	AA (forage ashed added back)
19	480	NC (no crop grown or forage added)
20	480	NA (no crop grown, ground forage from treatment 16 added back)

Table 3. Analysis of variance and comparisons for pH and electrical conductivity for Perkins and Stillwater soils.

		Perkins				Stillwater			
		Cycle				Cycle			
		1	2[Ψ]	3[#]	3[#]	1	2[Ψ]	3[#]	3[#]
		pH	pH	pH	EC (dS m^{-1})	pH	pH	pH	EC (dS m^{-1})
Source of variation	df	mean squares							
Replication	2	0.12@	0.009ns	0.04ns	5.41*	0.003ns	0.01ns	0.05ns	5.00ns
N rate	3	0.85**	2.92**	2.33**	73.92**	0.54**	1.73**	2.19*	159.91**
Methods	4	1.25**	0.38**	2.01**	138.65**	0.58**	0.29**	1.73*	224.89**
N rate*Method	12	0.09**	0.32**	0.29**	20.42**	0.02ns	0.15**	0.18**	20.75**
Residual	38	0.22	0.02	0.05	1.04	0.01	0.01	0.02	4.98
Contrasts: N rate linear	1	**	**	**	**	**	**	**	**
N rate quadratic	1	**	**	ns	ns	ns	ns	ns	ns

@, *, ** Significant at 0.1, 0.01 and 0.001 probability levels, respectively. [Ψ] cumulative applied N was 0, 320, 640 and 960 kg ha^{-1}. [#] cumulative applied N was 320, 880, 1,440 and 2,000 kg ha^{-1}. EC=Electrical conductivity. Saturation paste extract estimated from 1:5 soil:water extract.

Table 4. Effect of N rate on soil pH and electrical conductivity for Perkins and Stillwater soils (averaged over methods).

	Perkins				Stillwater			
	Cycle				Cycle			
	1	2[ψ]	3[#]	3[#]	1	2[ψ]	3[#]	3[#]
N rate, kg ha^{-1})	pH	pH	pH	EC (dS m^{-1})	pH	pH	pH	EC (dS m^{-1})
0	5.97	5.69	5.84	1.31	5.48	5.23	5.26	1.77
160	5.62	5.25	5.49	3.32	5.29	4.65	4.97	4.30
320	5.46	4.79	5.19	4.68	5.15	4.48	4.74	6.17
480	5.47	4.75	4.92	6.60	5.04	4.55	4.36	9.52
Contrasts:								
N rate linear 1	**	**	**	**	**	**	**	**
N rate quadratic1	**	**	ns	ns	ns	ns	ns	ns

ψ cumulative applied N was 0, 320, 640 and 960 kg ha^{-1}. # cumulative applied N was 320, 880, 1,440 and 2,000 kg ha^{-1}. EC=Electrical conductivity. Saturation paste extract estimated from 1:5 soil:water extract.

Table 5. Effect of forage management on soil pH and electrical conducty for Perkins and Stillwater soils (averaged over N rates).

	Perkins				Stillwater			
	Cycle				Cycle			
	1	2[ψ]	3[#]	3[#]	1	2[ψ]	3[#]	3[#]
Forage management	pH	pH	pH	EC (dS m^{-1})	pH	pH	pH	EC (dS m^{-1})
RR	5.83	5.06	5.51	1.72	5.40	4.75	5.05	2.21
AA	5.87	5.31	5.73	1.36	5.39	4.86	5.21	2.20
GA	5.87	5.31	5.70	1.39	5.41	4.90	5.06	4.48
NC	5.36	4.94	4.82	7.21	5.00	4.54	4.44	10.71
NA	5.20	4.99	5.04	8.12	5.00	4.59	4.41	9.62

RR = Forage removed and not added back; GA = Forage ground and added back; AA = Forage ashed and added back; NC = No crop grown or forage added; NA = No crop, ground forage from RR added back. ψ cumulative applied N was 0, 320, 640 and 960 kg ha^{-1}. # cumulative applied N was 320, 880, 1,440 and 2,000 kg ha^{-1}. EC= Electrical conductivity. Saturation paste extract estimated from 1:5 soil:water extract.

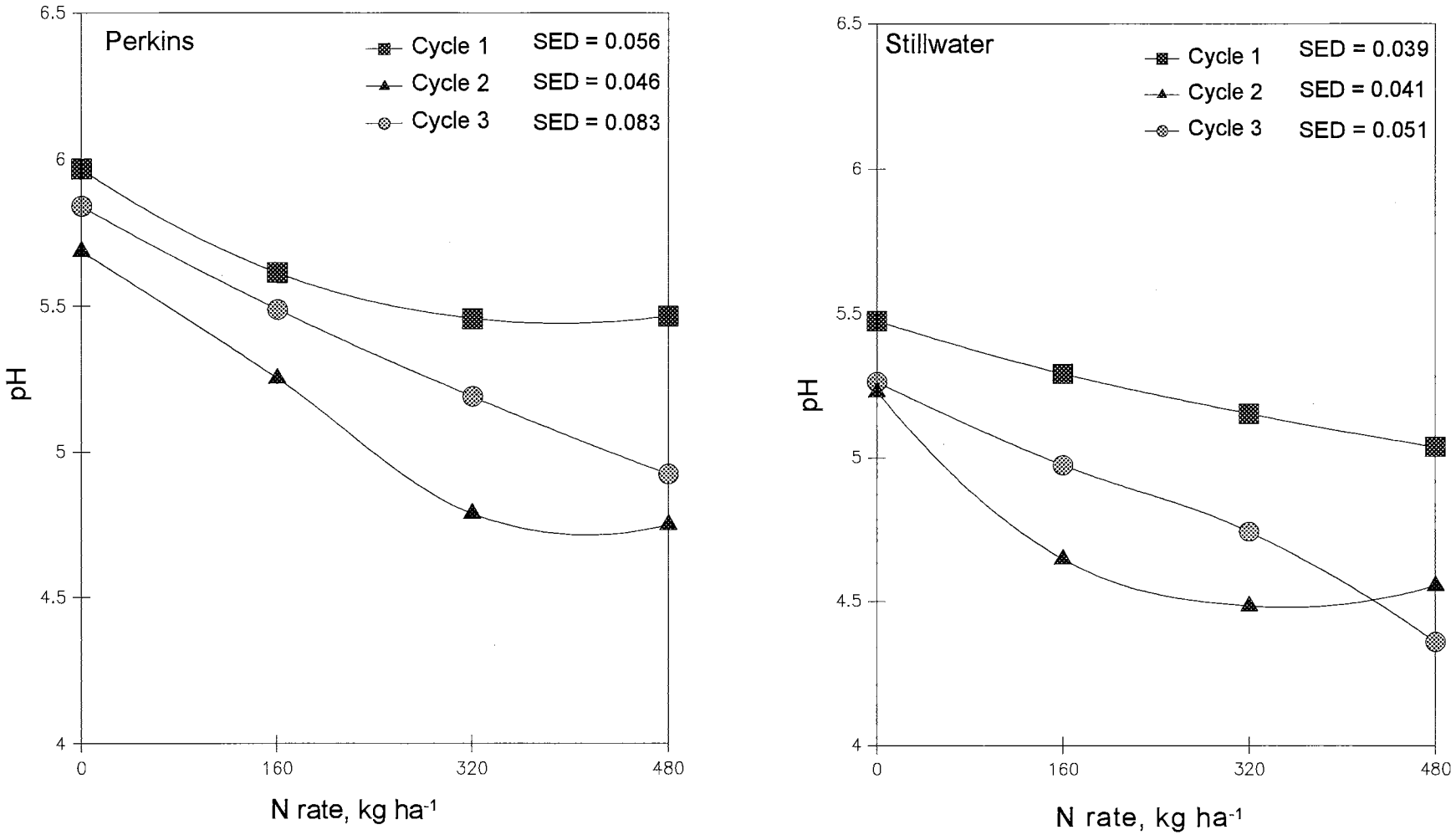

Figure 1. Effect of nitrogen rate on soil pH for Perkins and Stillwater soils. Cumulative N rates for cycle 2 were 0, 320, 640 and 960 kg ha^{-1} while for cycle 3 they were 320, 880, 1,440 and 2,000 kg ha^{-1}.

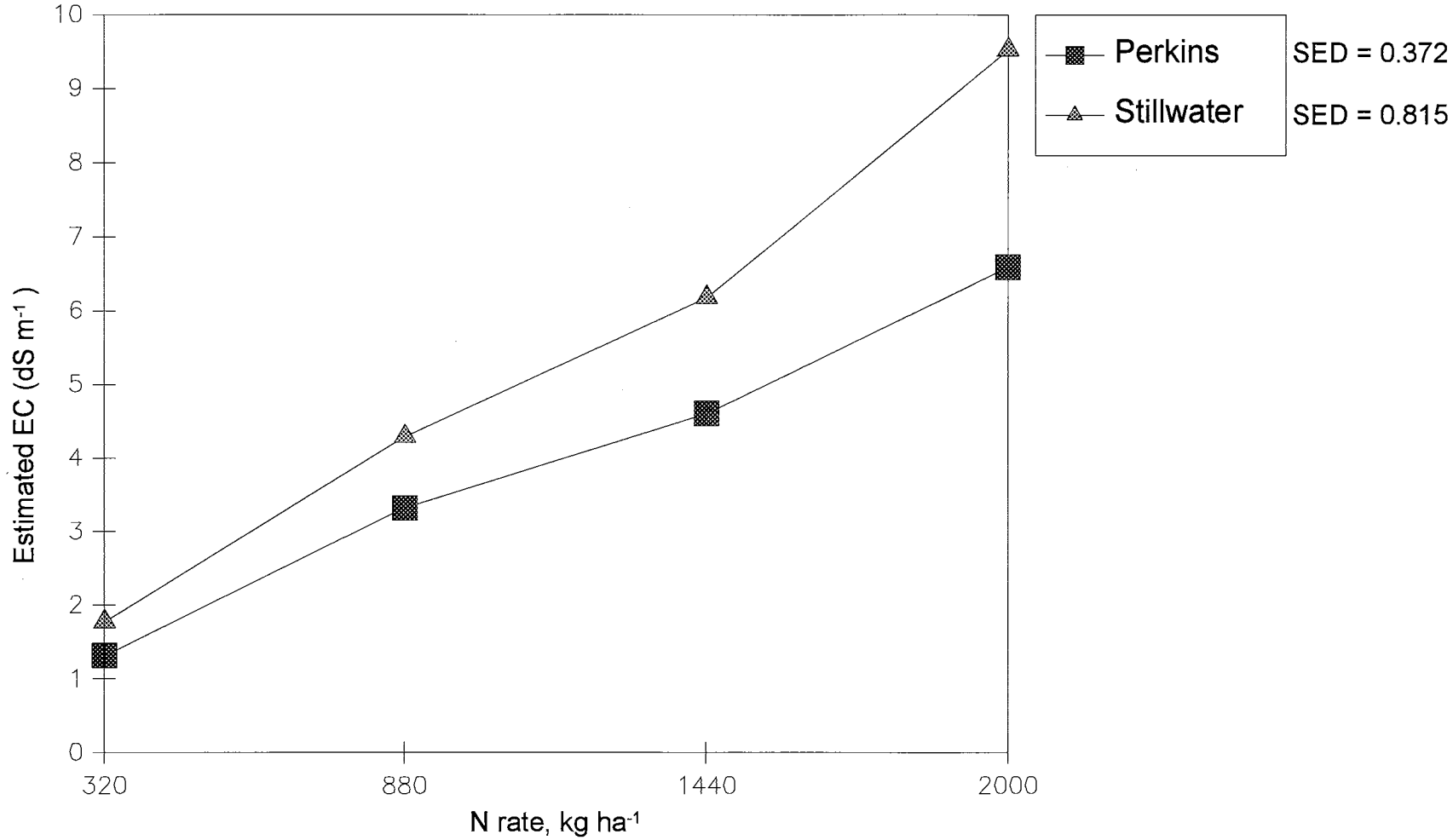

Figure 2. Effect of nitrogen rates on electrical conductivity of Perkins and Stillwater soils. Saturation paste extract estimated from 1:5 soil: water extract.

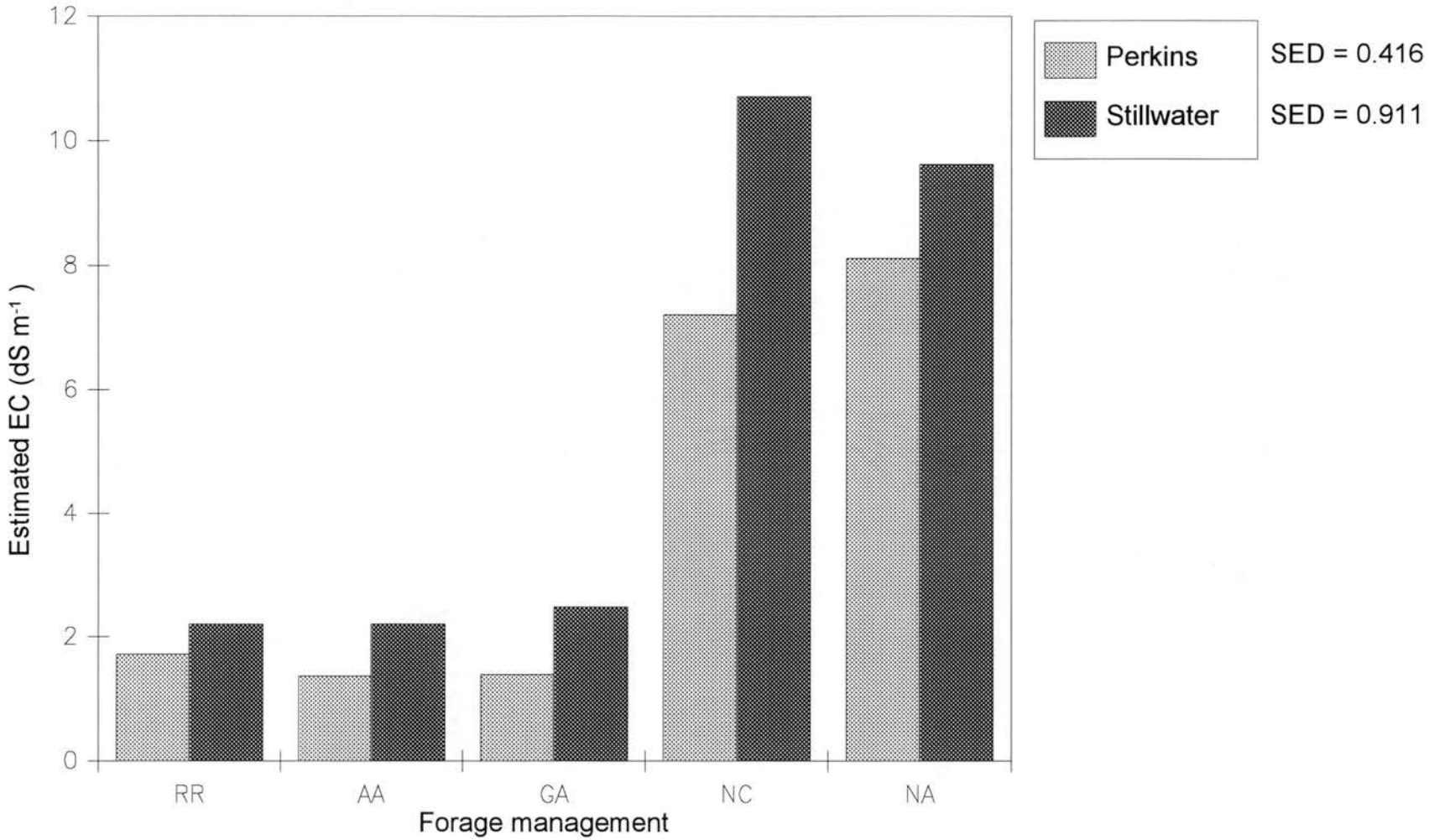

Figure 3. Effect of forage management on electrical conductivity of Perkins and Stillwater soils. Saturation paste extract estimate from 1:5 soil:water extract.

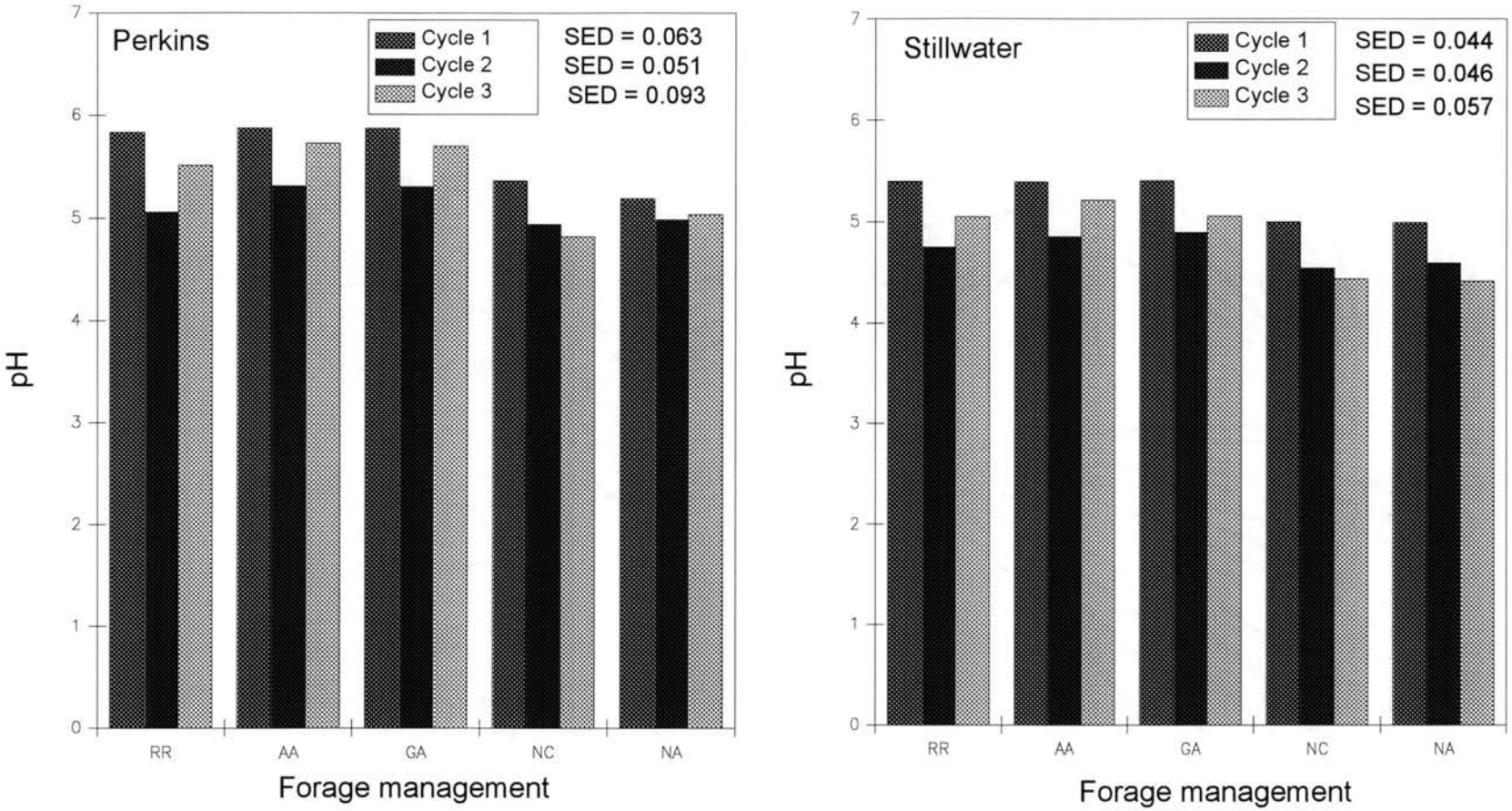

Figure 4. Effect of forage management on soil pH of Perkins and Stillwater soils. Cumulative Nrate for cycle 2 were 0, 320, 640 and 960 kg ha^{-1} while for cycle 3 were 320, 880, 1,440 and 2,000 kg ha^{-1}.

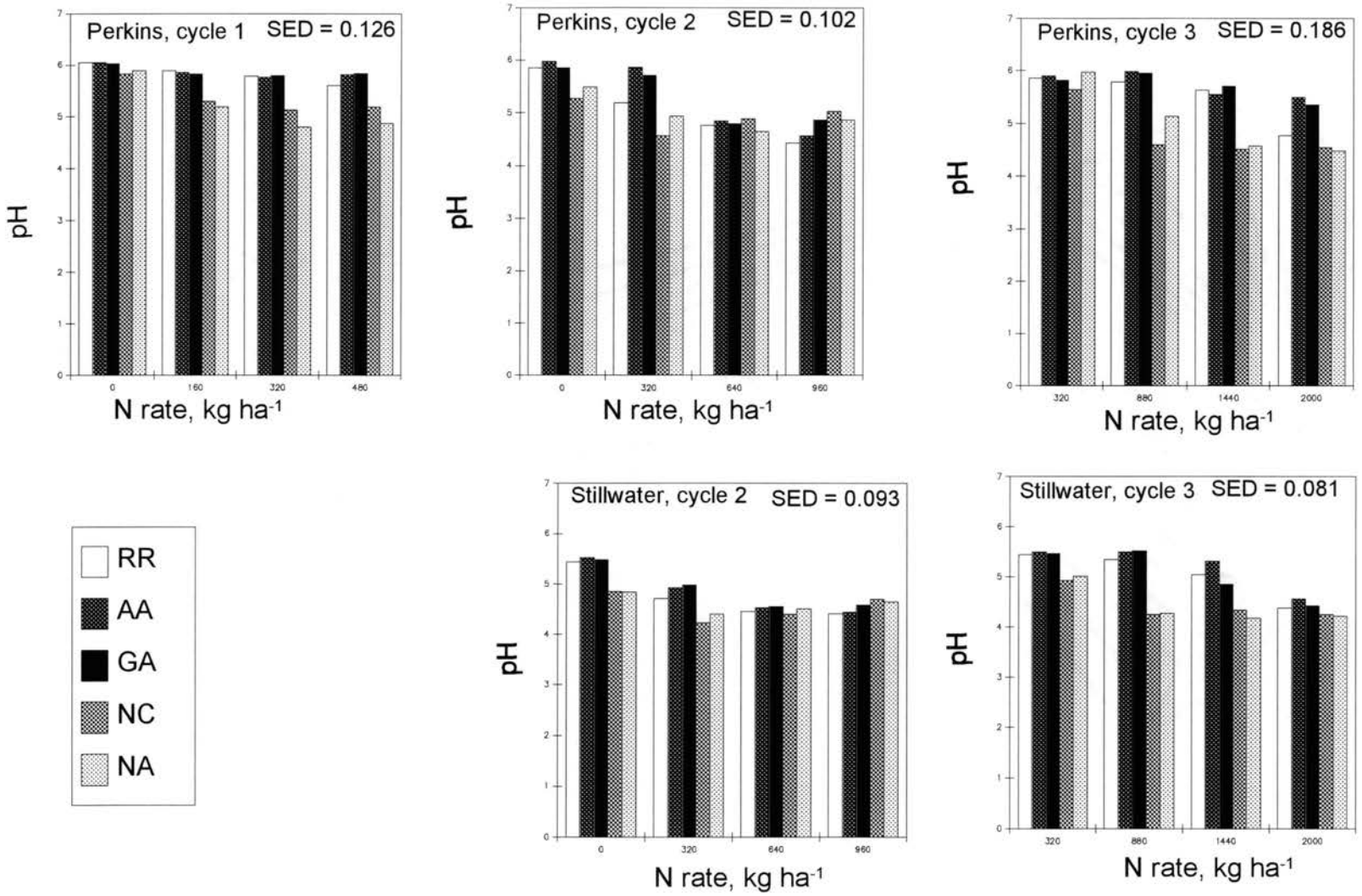

Figure 5. Effect of nitrogen rate and forage management on soil pH.

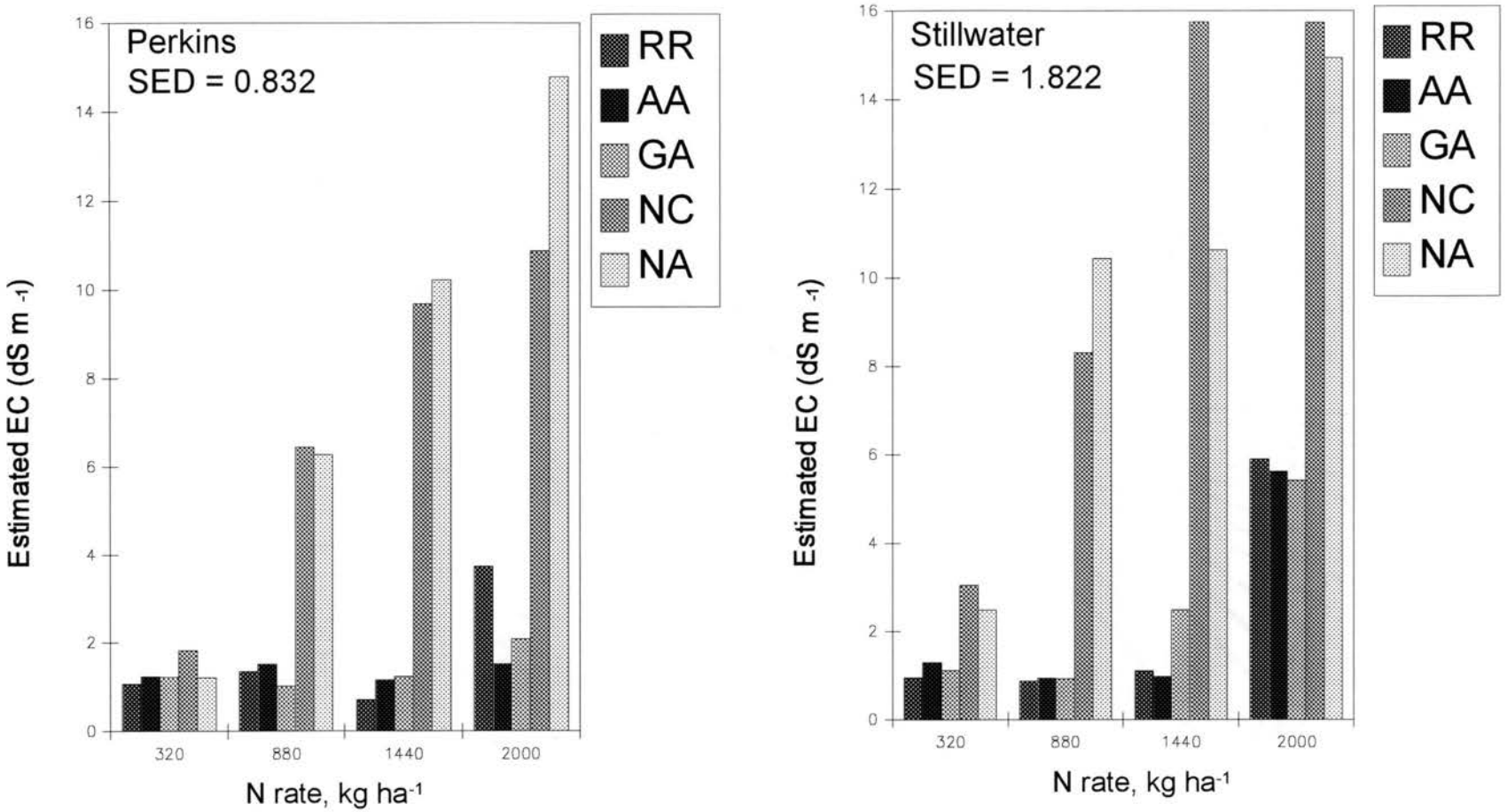

Figure 6. Effect of nitrogen rate and forage management on soil electrical conductivity of Perkins and Stillwater soils. Saturation paste extract estimated from 1:5 soil:water extract.

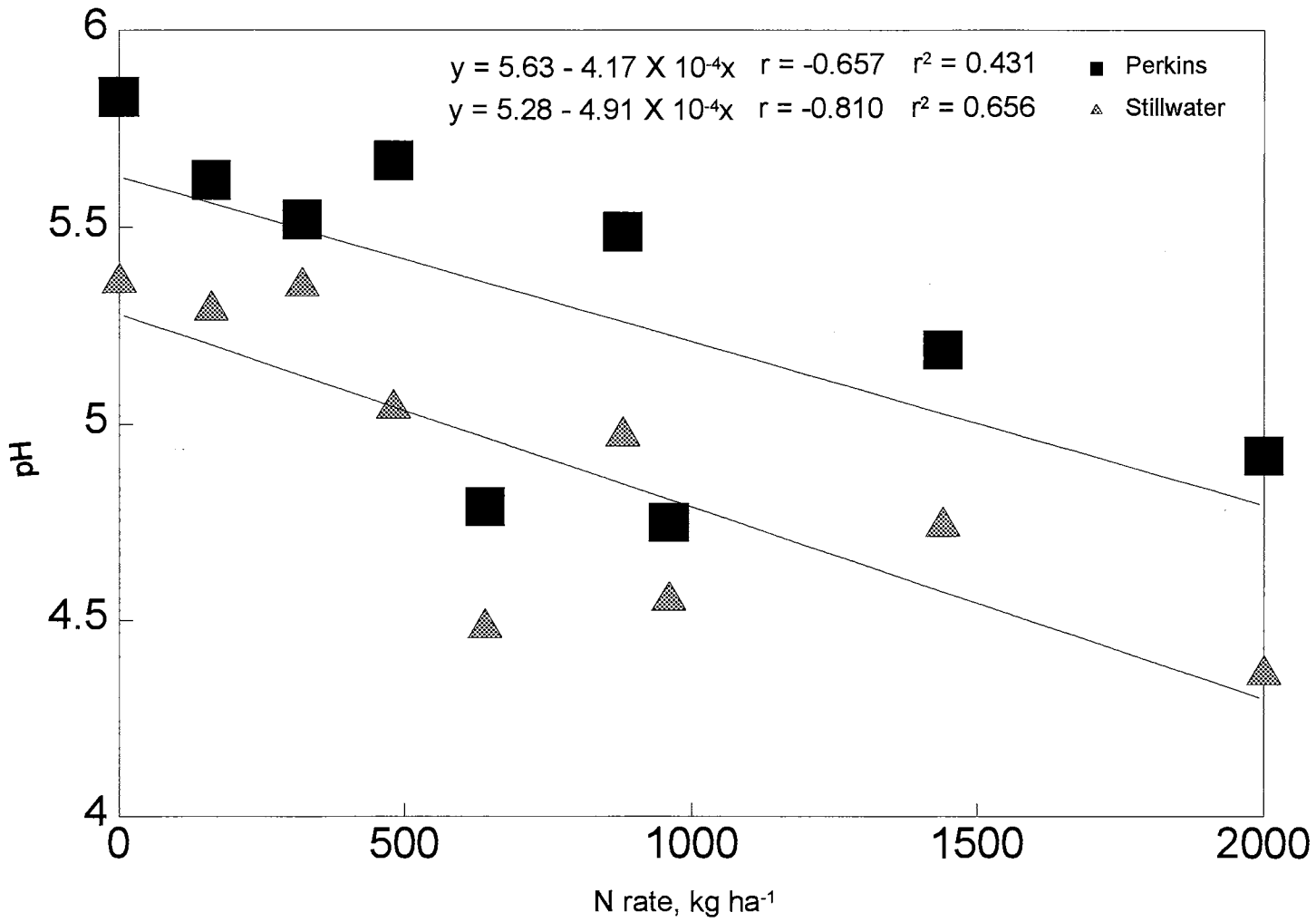

Figure 7. Effect of cumulative N on soil pH for Perkins and Stillwater soils.

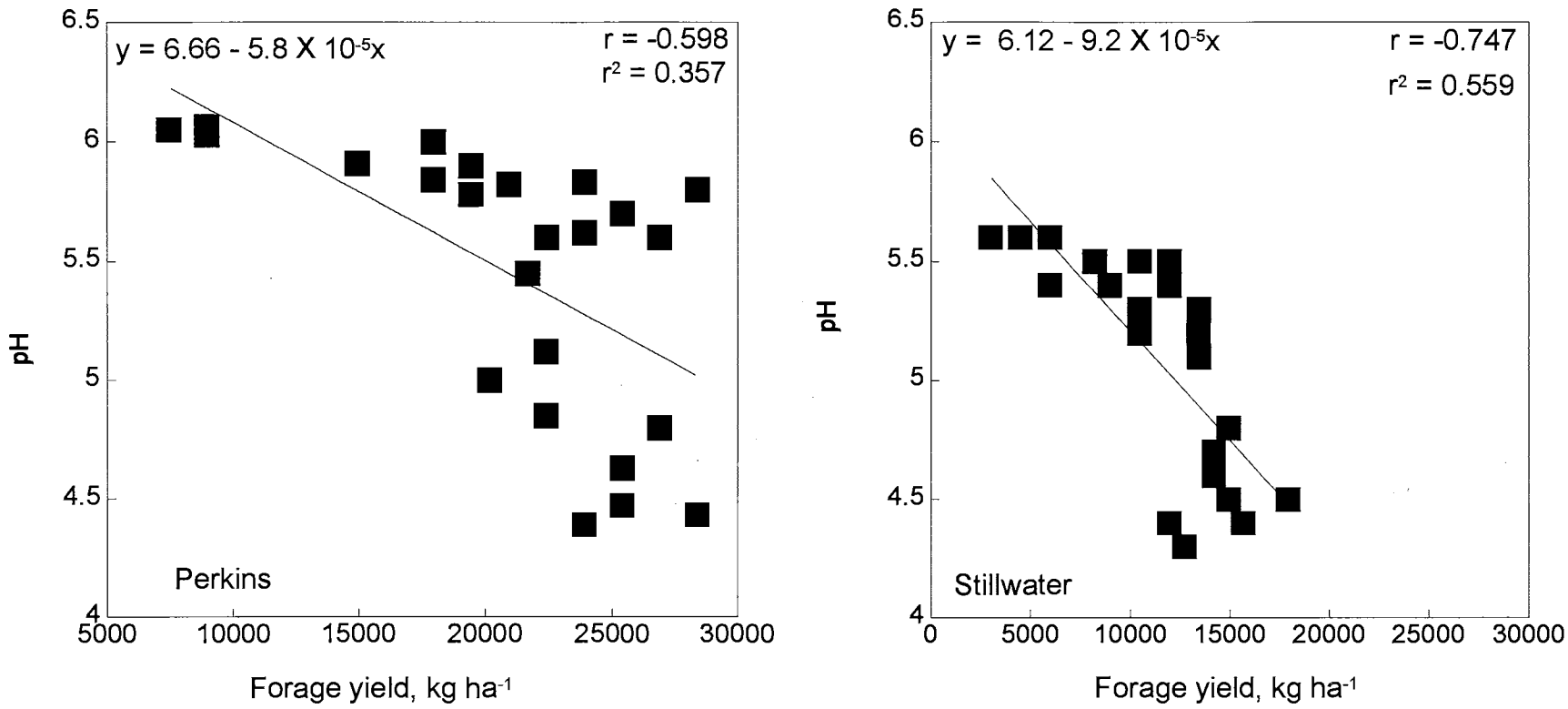

Figure 8. Effect of cumulative forage removal on soil pH of Perkins and Stillwater soils.

APPENDICES

APPENDIX - A

SAS PROGRAM FOR EFFECT OF NITROGEN LOSS IN WINTER WHEAT VARIETIES EXPERIMENT AT STILLWATER, OK 1994.

```
/* FPLOTWT = FORAGE PLOT WEIGHT IN LBS/45 FT2
PLANTING DATE OCTOBER 27, 1993
 HARVEST DATE AT MATURITY JULY 14, 1995
 FORAGE HARVEST AREA = 36" X 15' = 45 FT2
 GRAIN HARVEST AREA = 5 ROWS (9" SPACING) X 35 ' = 131.25FT2
FSWETWT = WET WEIGHT OF THE FORAGE (SUBSAMPLE)
FSDRYWT = DRY WEIGHT OF THE FORAGE (SAME SUBSAMPLE)
FYLDLBAC = DRY FORAGE YIELD, LB/AC
FYLDKGHA = DRY FORAGE YIELD, KG/HA
GPLOTWT = GRAIN PLOT WEIGHT IN LB/131.25 FT2
GYLDLBAC = DRY GRAIN YIELD, LB/AC
GYLDKGHA = DRY GRAIN YIELD, KG/HA
SPLOTWT = STRAW PLOT WEIGHT IN LB/131.25 FT2
SSWETWT = WET WEIGHT OF THE STRAW (SUBSAMPLE)
SSDRYWT = DRY WEIGHT OF THE STRAW (SAME SUBSAMPLE)
SYLDLBAC = DRY STRAW YIELD, LB/AC
SYLDKGHA = DRY STRAW YIELD, KG/HA
FN = TOTAL N ANALYSIS ON THE FORAGE
FNRLBAC = NITROGEN REMOVED IN THE FORAGE, LB/AC
FNRKGHA = NITROGEN REMOVED IN THE FORAGE, KG/HA
GN = TOTAL N ANALYSIS ON THE GRAIN
GNRLBAC = NITROGEN REMOVED IN THE GRAIN, LB/AC
GNRKGHA = NITROGEN REMOVED IN THE GRAIN, KG/HA
SN = TOTAL N ANALYSIS ON THE STRAW
SNRLBAC = NITROGEN REMOVED IN THE STRAW, LB/AC
SNRKGHA = NITROGEN REMOVED IN THE STRAW, KG/HA
NREMHAC = NITROGEN REMOVED AT HARVEST PER ACRE, LB/AC
NLOSSAC = NITROGEN LOSS BETWEEN FLOWERING AND HARVESTING PER ACRE
NREMHHA = TOTAL NITROGEN (GRAIN + STRAW) AT HARVEST, KG/HA
NO3_N   = NITRATE IN FORAGE
PROTEIN = % PROTEIN IN GRAINS (GN*5.7)
NUSE    = NITROGEN USE EFFICIENCY (GRAIN YIELD/N SUPPLY)
NUPE    = NITROGEN UPTAKE EFFICIENCY (TOTAL PLANT N/N SUPPLY)
NUTE = NITROGEN UTILIZATION EFFICIENCY (GRAIN YIELD/TOTAL PLANT N)
NTRG = FRACTION OF N TRANSLOCATED TO THE GRAIN (GRAIN YIELD/TOTAL PLANT N)
GWTN = GRAIN YIELD PER TOTAL GRAIN N (GRAIN YIELD/TOTAL GRAIN N)  */

DATA ONE; INFILE 'C:\WP51\FKA\LS94R.DAT' LRECL=350;
 INPUT REP NRATE VAR FPLOTWT FSWETWT FSDRYWT GPLOTWT GMC SPLOTWT
SSWETWT SSDRYWT SMC FN GN SN NO3_N;
DATA TWO; SET ONE;
LENGTH VARA $10;
 IF VAR= 1 THEN VARA = 'KARL';
 IF VAR =2 THEN VARA = 'HIGRP';
 IF VAR= 3 THEN VARA = 'P_2180';
 IF VAR =4 THEN VARA = 'LOGRP';
 IF VAR =5 THEN VARA = 'TAM_101';
 IF VAR= 6 THEN VARA = 'COMPOSITE';
 IF VAR =7 THEN VARA = 'CHISOLM';
 IF NRATE = 1 THEN NRATE ='0';
 IF NRATE = 2 THEN NRATE ='30';
 IF NRATE = 3 THEN NRATE ='60';
 IF NRATE = 4 THEN NRATE ='120';
FMC = ((FSWETWT - FSDRYWT)/FSWETWT);
FPLOTWT = FPLOTWT/.6;
FYLDLBAC = ((FPLOTWT*43560)/45)*(1-FMC);
FYLDKGHA = FYLDLBAC*1.12;
GYLDLBAC = ((GPLOTWT*43560)/131.25)*(1-(GMC/100));
GYLDKGHA = GYLDLBAC*1.12;
SYLDLBAC = ((SPLOTWT*43560)/131.25)*(1-(SMC/100));
SYLDKGHA = SYLDLBAC*1.12;
FNRLBAC = FYLDLBAC*(FN/100);
```

```
FNRKGHA = FYLDKGHA *(FN/100);
GNRLBAC = GYLDLBAC*(GN/100);
GNRKGHA = GYLDKGHA *(GN/100);
SNRLBAC = SYLDLBAC*(SN/100);
SNRKGHA = SYLDKGHA *(SN/100);
NREMHAC = GNRLBAC + SNRLBAC;
NREMHHA = GNRKGHA + SNRKGHA;
NLOSSAC = FNRLBAC - NREMHAC;
NLOSSHA = FNRKGHA - NREMHHA;
PROTEIN = GN*5.7;
NUSE    = GYLDKGHA/NRATE;
NUPE    = NREMHHA/NRATE;
NUTE    = GYLDKGHA/NREMHHA;
NTRG    = GNRKGHA/NREMHHA;
GWTN    = GYLDKGHA/GNRKGHA;
/* PROC PRINT;
PROC  GLM;
 CLASSES REP NRATE VARA;
 MODEL FYLDKGHA GYLDKGHA SYLDKGHA FNRKGHA GNRKGHA SNRKGHA
 NREMHHA NLOSSHA PROTEIN NUSE  NUPE NUTE NTRG GWTN = REP NRATE
VARA NRATE*VARA;
 CONTRAST 'N_LIN' NRATE -3 -1 1 3;
 CONTRAST 'N_QUA' NRATE 1 -1 -1 1;
 MEANS NRATE VARA NRATE*VARA;
 MEANS NRATE VARA/SNK; */

PROC  GLM;
 CLASSES REP NRATE VARA;
 MODEL NUSE  NUPE = REP NRATE VARA NRATE*VARA;
 CONTRAST 'N_LIN' NRATE -1 0 1;
 CONTRAST 'N_QUA' NRATE -1 2 -1 ;
 MEANS NRATE VARA NRATE*VARA;
 MEANS NRATE VARA/SNK;
RUN;

  FILENAME GRAFOUT 'C:\WP51\FKA\NLOSSHA.HGL';
  GOPTIONS NODISPLAY GSFMODE=REPLACE DEVICE=HPLJS2
  GSFNAME=GRAFOUT GWAIT=10 FBY=XSWISS HBY = 1.75
GOUTTYPE=DEPENDENT;
DATA ONE; SET TWO; PROC SORT; BY VARA;
PROC RSREG DATA = ONE OUT = TWO;
  MODEL NLOSSHA = FN FYLDKGHA/PREDICT;
PROC G3GRID DATA = TWO OUT = THREE;
  GRID FYLDKGHA*FN = NLOSSHA;
PROC G3D DATA = THREE GOUT = NEW1;
  PLOT FYLDKGHA*FN=NLOSSHA;
```

APPENDIX - B

SAS PROGRAM FOR EFFECT OF NITROGEN LOSS IN WINTER WHEAT VARIETIES EXPERIMENT AT PERKINS, OK 1995.

```
/* FORAGE HARVEST AREA AT FLOWERING = 18" X 10' = 15 FT2 */
/* PLANTING DATE OCTOBER 24, 1994 */
/* FORAGE HARVEST DATE AT ANTHESIS APRIL 24, 1995 */
/* FORAGE HARVEST DATE POST-ANTHESIS MAY 8, 1995 */
/* HARVEST DATE AT MATURITY, VARIETY TRIAL, PERKINS, OK, JUNE 14, 1995 */
/* GRAIN AND STRAW HARVEST AREA = 45" X 30 ' = 112.5 FT2 */
/* FFPLOTWT = FORAGE PLOT WEIGHT AT FLOWERING IN LBS/15 FT2
FFSWETWT = WET WEIGHT OF THE FORAGE AT FLOWERING (SUBSAMPLE)
FFSDRYWT = DRY WEIGHT OF THE FORAGE AT FLOWERING (SAME SUBSAMPLE)
FFYDLBAC = DRY FORAGE YIELD AT FLOWERING AT FLOWERING, LB/AC
FFYDKGHA = DRY FORAGE YIELD AT FLOWERING, KG/HA
PFPLOTWT = FORAGE PLOT WEIGHT AT POST FLOWERING IN LBS/15 FT2
PFSWETWT = WET WEIGHT OF THE FORAGE AT POST FLOWEERING (SUBSAMPLE)
PFSDRYWT = DRY WEIGHT OF THE FORAGE AT POST FLOWERING (SAME
SUBSAMPLE)
PFYDLBAC = DRY FORAGE YIELD AT POST FLOWERING, LB/AC
PFYDKGHA = DRY FORAGE YIELD AT POST FLOWERING, KG/HA
GPLOTWT = GRAIN PLOT WEIGHT IN LB/112.5 FT2
GYDLBAC = DRY GRAIN YIELD, LB/AC
GYDKGHA = DRY GRAIN YIELD, KG/HA
SPLOTWT = STRAW PLOT WEIGHT IN LB/112.5 FT2
SSWETWT = WET WEIGHT OF THE STRAW (SUBSAMPLE)
SSDRYWT = DRY WEIGHT OF THE STRAW (SAME SUBSAMPLE)
SYLDLBAC = DRY STRAW YIELD, LB/AC
SYLDKGHA = DRY STRAW YIELD, KG/HA
FFN = TOTAL N ANALYSIS ON THE FORAGE AT FLOWERING
FFNRLBAC = NITROGEN REMOVED IN THE FORAGE AT FLOWERING, LB/AC
FFNRKGHA = NITROGEN REMOVED IN THE FORAGE AT FLOWERING, KG/HA
PFN = TOTAL N ANALYSIS ON THE FORAGE AT POST FLOWERING
PFNRLBAC = NITROGEN REMOVED IN THE FORAGE AT POST FLOWERING, LB/AC
PFNRKGHA = NITROGEN REMOVED IN THE FORAGE AT POST FLOWERING, KG/HA
GN = TOTAL N ANALYSIS ON THE GRAIN
GNRLBAC = NITROGEN REMOVED IN THE GRAIN, LB/AC
GNRKGHA = NITROGEN REMOVED IN THE GRAIN, KG/HA
SN = TOTAL N ANALYSIS ON THE STRAW
SNRLBAC = NITROGEN REMOVED IN THE STRAW, LB/AC
SNRKGHA = NITROGEN REMOVED IN THE STRAW, KG/HA
NREMHAC = NITROGEN REMOVED AT HARVEST PER ACRE, LB/AC
NLOSSAC = NITROGEN LOSS BETWEEN FLOWERING AND HARVESTING PER ACRE
NREMHHA = TOTAL NITROGEN (GRAIN + STRAW) AT HARVEST, KG/HA
ANLOSSHA = NITROGEN LOSS BETWEEN ANTHESIS AND POST-ANTHESIS, KG/HA
PROTEIN = % PROTEIN IN GRAINS (GN*5.7)
NUSE = NITROGEN USE EFFICIENCY (GRAIN YIELD/N SUPPLY)
NUPE = NITROGEN UPTAKE EFFICIENCY (TOTAL PLANT N/N SUPPLY)
NUTE = NITROGEN UTILIZATION EFFICIENCY (GRAIN YIELD/TOTAL PLANT N)
NTRG = FRACTION OF N TRANSLOCATED TO THE GRAIN (GRAIN YIELD/TOTAL PLANT N)
GWTN = GRAIN YIELD PER TOTAL GRAIN N (GRAIN YIELD/TOTAL GRAIN N) */
DATA ONE; INFILE 'C:\WP51\FKA\95NXV.DAT' LRECL= 350;
INPUT HARSQNCE REP TRT NRATE VARIETY $ FFPLOTWT FFSWETWT FFSDRYWT
PFPLOTWT PFSWETWT PFSDRYWT GPLOTWT GMC SPLOTWT SSWETWT FFN PFN SN GN;
 FFMC = ((FFSWETWT - FFSDRYWT)/FFSWETWT);
 PFMC = ((PFSWETWT - PFSDRYWT)/PFSWETWT);
 FFYDLBAC = ((FFPLOTWT*43560)/15)*(1-FFMC);
 PFYDLBAC = ((PFPLOTWT*43560)/15)*(1-PFMC);
 FFYDKGHA = FFYDLBAC*1.12;
 FFYDMGHA = FFYDKGHA/1000;
 PFYDKGHA = PFYDLBAC*1.12;
 PFYDMGHA = PFYDKGHA/1000;
 GYDLBAC = ((GPLOTWT*43560)/112.5)*(1-(GMC/100));
 GYDKGHA = GYDLBAC*1.12;
 GYDMGHA = GYDKGHA/1000;
 SYDLBAC = ((SPLOTWT*43560)/112.5);
```

```
SYDKGHA = SYDLBAC*1.12;
SYDMGHA = SYDKGHA/1000;
FFNRKGHA = FFYDKGHA *(FFN/100);
FFNRMGHA = FFNRKGHA/1000;
PFNRKGHA = PFYDKGHA *(PFN/100);
PFNRMGHA = PFNRKGHA/1000;
GNRKGHA = GYDKGHA *(GN/100);
GNRMGHA = GNRKGHA/1000;
SNRKGHA = SYDKGHA *(SN/100);
SNRMGHA = SNRKGHA/1000;
NREMKGHA = GNRKGHA + SNRKGHA;
NREMMGHA = NREMKGHA/1000;
FNLOSSHA = FFNRKGHA - NREMKGHA;
PNLOSSHA = PFNRKGHA - NREMKGHA;
ANLOSSHA = FNLOSSHA - PNLOSSHA;
PROTEIN = GN*5.7;
NUSE = GYDKGHA/NRATE;
NUPE = NREMKGHA/NRATE;
NUTE = GYDKGHA/NREMKGHA;
NTRG = GNRKGHA/NREMKGHA;
GWTN = GYDKGHA/GNRKGHA;
PROC PRINT;
DATA TWOO; SET ONE;
IF TRT > 32 THEN DELETE;
PROC GLM;
CLASSES REP NRATE VARIETY;
 MODEL FFYDKGHA FFYDMGHA PFYDKGHA PFYDMGHA GYDKGHA GYDMGHA
SYDKGHA SYDMGHA FFNRKGHA FFNRMGHA PFNRKGHA PFNRMGHA GNRKGHA NRMGHA
SNRKGHA SNRMGHA NREMKGHA NREMMGHA FNLOSSHA PNLOSSHA ANLOSSHA PROTEIN
NUTE NTRG GWTN = REP NRATE VARIETY NRATE*VARIETY;
CONTRAST 'N_LIN' NRATE -3 -1 1 3;
CONTRAST 'N_QUA' NRATE 1 -1 -1 1;
MEANS NRATE VARIETY NRATE*VARIETY;
MEANS NRATE VARIETY/SNK;
DATA TWO; SET TWOO;
PROC GLM;
CLASSES REP NRATE VARIETY;
MODEL NUSE NUPE = REP NRATE VARIETY NRATE*VARIETY;
CONTRAST 'N_LIN' NRATE -1 0 1;
CONTRAST 'N_QUA' NRATE -1 2 -1;
MEANS NRATE VARIETY NRATE*VARIETY;
MEANS NRATE VARIETY/SNK;
DATA THREE; SET ONE;
PROC GLM;
CLASSES REP TRT;
MODEL FFYDKGHA FFYDMGHA PFYDKGHA PFYDMGHA GYDKGHA GYDMGHA SYDKGHA
SYDMGHA FFNRKGHA FFNRMGHA PFNRKGHA PFNRMGHA GNRKGHA GNRMGHA
SNRKGHA SNRMGHA NREMKGHA NREMMGHA FNLOSSHA PNLOSSHA ANLOSSHA
PROTEIN NUTE NTRG GWTN = REP TRT;
CONTRAST '18 VS 36' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0;
CONTRAST '19 VS 37' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0;
CONTRAST '20 VS 38' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1;
CONTRAST '30 VS 33' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 1 0 0 -1 0 0 0 0 0;
CONTRAST '31 VS 34' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 1 0 0 -1 0 0 0 0;
CONTRAST '32 VS 35' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 1 0 0 -1 0 0 0;
MEANS TRT;
DATA FOUR; SET ONE;
 PROC GLM;
 CLASSES REP TRT;
 MODEL NUSE NUPE = REP TRT;
CONTRAST '18 VS 36' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0
```

```
0 0 0 0 0 0 0 0 0 0 -1 0 0;
 CONTRAST '19 VS 37' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 0 0 0 0 0 0 0 0 0 0 -1 0;
 CONTRAST '20 VS 38' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
0 0 0 0 0 0 0 0 0 0 0 0 -1;
 CONTRAST '30 VS 33' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 -1 0 0 0 0 0;
 CONTRAST '31 VS 34' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 0 0 -1 0 0 0 0;
 CONTRAST '32 VS 35' TRT 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 1 0 0 -1 0 0 0;
 MEANS TRT;

 FILENAME GRAFOUT 'C:\WP51\FKA\FNLOSSHA.HGL';
 GOPTIONS NODISPLAY GSFMODE=REPLACE DEVICE=HPLJS2
 GSFNAME=GRAFOUT GWAIT=10 FBY=XSWISS HBY = 1.75
GOUTTYPE=DEPENDENT;
DATA ONE; SET TWOO; PROC SORT; BY VARIETY;
PROC RSREG DATA = ONE OUT = TWO;
  MODEL FNLOSSHA = FFN FFYDKGHA/PREDICT;
PROC G3GRID DATA = TWO OUT = THREE;
  GRID FFYDKGHA*FFN = FNLOSSHA;
PROC G3D DATA = THREE GOUT = NEW1;
  PLOT FFYDKGHA*FFN=FNLOSSHA;
RUN;
```

APPENDIX - C

SAS PROGRAM FOR GLUTAMINE SYNTHETASE ACTIVITY IN WINTER WHEAT VARIETIES EXPERIMENT AT PERKINS, OK 1995.

```
DATA ONE; INFILE 'C:\WP51\FKA\ENZYME.DAT' LRECL= 350;
INPUT REP NRATE VARIETY $ GSACT1 GSACT2 PROTEIN SPECACT AMMONIUM NH4 FFN PFN SN GN FNLOSS
NUSE NUPE NTRG GWTN GYDKGHA FNKGHA GNKGHASNKGHA NREKGHA NUTE SYDKGHA;
NH4 = (AMMONIUM*18);

DATA TWO; SET ONE;
PROC GLM;
        CLASSES REP NRATE VARIETY;
        MODEL PROTEIN SPECACT AMMONIUM FFN PFN SN GN FNLOSS NUSE NUPE NUTE NTRG GWTN
GYDKGHA FNKGHA GNKGHA SNKGHA NREKGHA= REP NRATE VARIETY NRATE*VARIETY;
        CONTRAST 'HIGRP_VS_LOGRP' VARIETY 0 0 1 0 -1 0 0 0;
        CONTRAST 'HIGRP_VS_COMPOSITE' VARIETY 0 1 -1 0 0 0 0 0;
        CONTRAST 'LOGRP_VS_COMPOSITE' VARIETY 0 1 0 0 -1 0 0 0;
        CONTRAST 'HIGRP AND LOGRP_VS_COMPOSITE' VARIETY 0 2 -1 0 -1 0 0 0;
MEANS NRATE VARIETY NRATE*VARIETY;
MEANS VARIETY/SNK;

PROC CORR;
        VAR PROTEIN SPECACT AMMONIUM NH4 FFN PFN SN GN FNLOSS NUSE
        NUPE NUTE NTRG GWTN GYDKGHA FNKGHA GNKGHA SNKGHA  NREKGHA;
RUN;
```

SAS PROGRAM FOR EFFECT OF NITROGEN FERTILIZATION AND CATION REMOVAL ON SOIL pH EXPERIMENT AT STILLWATER, OK 1995.

```
/* RR = FORAGE REMOVED AND NOT RETURNED
 AA = ASHED FORAGE RETURNED
 GA = GROUND FORAGE RETURNED
 NC = NO CROP, NOTHING ADDED
 NA = NO CROP, ASHED FORAGE FROM RR ADDED */

DATA ONE;
INPUT CYCLE REP NRATE METHOD $ PH ECONDUCT;
ECDS=ECONDUCT*14.9;
CARDS;
PROC PRINT;
DATA TWO; SET ONE;
 IF CYCLE > 1 THEN DELETE;
PROC GLM;
CLASSES REP NRATE METHOD;
MODEL PH = REP NRATE METHOD NRATE*METHOD;
 CONTRAST 'N-LIN' NRATE -3 -1 1 3;
 CONTRAST 'N-QUA' NRATE 1 -1 -1 1;
MEANS NRATE METHOD NRATE*METHOD;
MEANS NRATE METHOD/SNK;
RUN;

DATA THREE; SET ONE;
 IF CYCLE='1' OR CYCLE='3' THEN DELETE;
PROC PRINT;
PROC GLM;
CLASSES REP NRATE METHOD;
MODEL PH = REP NRATE METHOD NRATE*METHOD;
 CONTRAST 'N-LIN' NRATE -3 -1 1 3;
 CONTRAST 'N-QUA' NRATE 1 -1 -1 1;
MEANS NRATE METHOD NRATE*METHOD;
MEANS NRATE METHOD/SNK;
RUN;

DATA FOUR; SET ONE;
 IF CYCLE < 3 THEN DELETE;
PROC PRINT;
PROC GLM;
CLASSES REP NRATE METHOD;
MODEL PH ECDS = REP NRATE METHOD NRATE*METHOD;
 CONTRAST 'N-LIN' NRATE -3 -1 1 3;
 CONTRAST 'N-QUA' NRATE 1 -1 -1 1;
MEANS NRATE METHOD NRATE*METHOD;
MEANS NRATE METHOD/SNK;
RUN;
```

APPENDIX - E

ESTIMATION OF ELECTRICAL CONDUCTIVITY IN 1:5 SOIL EXTRACTS FOR PERKINS AND STILLWATER SOILS.

Perkins soil:	Stillwater soil:
bulk density = 1.45 g cm^{-3}	1.40 g cm^{-3}
particle density = 2.65 g cm^{-3}	2.65 g cm^{-3}
% solids: (1.45/2.65)*100 = 55%	(1.40/2.65)*100 = 53%
% porosity:100 - 55 = 45%	100- 53 = 47%
100 cm^3 soil = 45 cm^3 voids	47 cm^3 voids
At saturation soil has: 45 mls water	47 mls water
145 g soil has 45 ml water	140 g soil has 47 ml water
1 soil:5 water (w:v):145 g soil =725 ml water	140 g soil =700 ml water
Dilution for comparing soil to 1:5: (725/45) = 16.1	(700/47) = 14.9
Extract conductivity equivalent to 4 mmhos/cm:	
(4/16.1) = 0.25 mmhos/cm	(4/14.9) = 0.27 mmhos/cm

VITA

Fred Kaburu Kanampiu

Candidate for the Degree of

Doctor of Philosophy

Thesis: I. EFFECT OF NITROGEN RATE ON PLANT NITROGEN LOSS IN WINTER WHEAT VARIETIES
II. GLUTAMINE SYNTHETASE ACTIVITY IN WINTER WHEAT VARIETIES
III. EFFECT OF NITROGEN FERTILIZATION AND CATION REMOVAL ON SOIL pH

Major Field: Soil Science

Biographical:

Personal Data: Born in Meru, Kenya on November 1, 1960, son of Luciano and Rugina Kanampiu. Married to Sally Karimi Kanampiu; a son , Vernon Murimi Kanampiu, was born April 27, 1993.

Education: Graduated from Njiri's High School, Murang'a, Kenya, December, 1980; received the Bachelor of Science Degree in Agriculture from University of Nairobi, Nairobi, Kenya, July , 1985; received the Master of Science Degree in Agronomy from University of Nairobi, Nairobi, Kenya, December, 1988; and completed the requirements for the Doctor of Philosophy Degree in Soil Science from Oklahoma State University, Stillwater, Oklahoma, in December, 1995.

Professional experience: Deputy headmaster and teacher Kinoro Secondary School, Meru, Kenya. Jan-Dec, 1981. Agricultural officer, Ministry of Agriculture, Kenya 1987-1988. Research scientist, Kenya Agricultural Research Institute 1988-1992. Graduate student, Department of Agronomy, Oklahoma State University, January,1993-present.

Member: Crop Science Society of Kenya. African Soil Science Society. American Society of Agronomy. Soil Science Society of America. Crop Science Society of America. Sigma Xi.