AN ABSTRACT OF THE THESIS OF

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A study of mineral nitrogen forms (ammonium and nitrate) in a native prairie and reseeded old fields was conducted from January 1986 through December 1986. All study sites were located on the Kenoma soil series. Samples were taken from each site monthly and analyzed using the microdiffusion procedure of Keeney and Nelson (1982). The most probable number of Nitrosonmonas and Total Plate Count were also determined for each sample. A nitrification potential experiment was conducted in which there was minimal disturbance to the soil and vegetation.

Results from the microdiffusion tests indicated less than 1.0 ppm of ammonium or nitrate to be present in each of the study sites. The most probable number of Nitrosomonas was also low at all sites, though a decreasing trend from the most recently reseeded site to the native prairie was found. With the addition of ammonium, the amount of nitrate and number of Nitrosomonas increased only slightly. An increase in ammonium was detected, possibly due to mineralization of organic nitrogen. Overall biomass production was much lower in the control prairie site than that of a similar prairie which had not been heavily grazed. This suggests that grazing could be influencing the productivity and the nitrogen pool present. However, the low level of nitrogen present and small population of Nitrosomonas on all sites suggest the climax prairie grasses are affecting the forms of nitrogen.

NITROGEN FORMS IN RESEEDED KANSAS FARMLAND

A Thesis

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INTRODUCTION

Nitrogen is the key element in plant growth. Its presence is essential for the formation of plant proteins, chlorophyll, nucleic acids, and other plant substances (Donahue et al 1983). Although about 80 % of the Earth's atmosphere is nitrogen in the form of N gas, this form cannot be utilized by most plants. It must first undergo fixation, and often nitrification by micro-organisms in the soil (Gray and Williams 1971). Nitrogen in the soil itself may be present in various forms. These include the nitrogen present in organic matter; mineral nitrogen in the soil solution and on exchange sites; nitrogen in plant residues in the soil; ammonium fixed in clay minerals; and the gaseous nitrogen in the soil's atmosphere (Barber 1984). Through microbial metabolism, organic nitrogen is converted to mineral or inorganic nitrogen ions which may be utilized by plants (Alexander 1977).

Nitrogen as a plant nutrient is unique in that it may occur as a cation, ammonium (NH_4^+), or an anion, nitrate (NO_3^-) (Donahue et al 1983). The clay mineral fraction found in many soils is crystalline in structure and has a net negative charge, thus allowing for adsorption of the ammonium (NH_4^+) cations (Salisbury and Ross 1985). These ammonium cations may be rapidly oxidized to nitrate anions through the activity of two autotrophic bacteria: Nitrosomonas and Nitrobacter (Gray and Williams 1971). Nitrosomonas oxidizes ammonium to nitrite and Nitrobacter oxidizes nitrite to nitrate. The nitrate anions, repelled by the negatively charged soil colloids may be lost by leaching of the soil (Salisbury and Ross 1985).

Previous studies have indicated that the nitrate form of nitrogen is associated mainly with agriculture and early successional stages of prairie; whereas the ammonium form of nitrogen is more common in

natural vegetation near climax. Rice and Pancholy (1972) and Rice (1984) have done studies on three vegetation types in Oklahoma, the oak - pine forest, the post oak - blackjack oak forest, and the tall grass prairie. Their work indicates the amount of nitrate present in the first successional stage was greatest, it was intermediate in the second successional stage, and lowest in the climax stage for all three communities. Conversely, the amount of ammonium was the lowest in the first successional stage and highest in the climax stand. They suggested that climax prairie plants suppress nitrification and that this suppression is selectively advantageous because it conserves nitrogen and energy.

Haines (1977) examined nitrogen uptake in two old fields, in a pine plantation, and in a hardwood forest in Georgia. His study concluded that plants from the early successional stage preferentially utilize nitrate while those from later stages use ammonium. Montes and Christensen (1979) examined characteristics of nitrification in three early stages of succession in North Carolina: old fields, loblolly pine forest, and oak - hickory forests. Soil incubation experiments carried out in their lab indicated the rate of nitrate production to be highest in the old fields and lowest in the pine soils. Various successional stages in mine spoil soils of North Dakota were studies by Lodhi (1979). Again nitrification decreased from pioneer to climax stages.

Not all data have supported the hypotehsis of nitrification being progressively inhibited in the course of succession. Robertson and Vitousek (1981), using soil from the Indiana Dunes on the southern edge of Lake Michigan as their primary sere and soil from the New Jersey

Peidmont as their secondary sere, found no clear evidence for a successional trend in the inhibition of nitrification. Lamb (1980) examined five successional stages of a sub tropical rain forest and found little difference in ammonium and nitrate concentration. However, in each older successional stage increasing amounts of nitrate nitrogen were found. He proposed that rather than due to succession, nitrification is dependent on the availability of ammonium nitrogen.

To determine if the availability of ammonium is an influencing factor, nitrification potential studies have been performed by several researchers (Robertson and Vitousek 1981; Montes and Christensen 1979; Rice and Pancholy 1972). In these studies soil samples were taken from the field to a laboratory where excess amounts of ammonium were added and nitrate production determined. Robertson and Vitousek sieved the soil and incubated the samples in polyethylene cups. Montes and Christensen also used plastic cups for incubation, while Rice and Pancholy used a solution culture. These methods all involve a major disturbance of the soil which itself had been found to increase nitrification (Vitousek et al 1979).

The relationship between the Most Probable Number of Nitrosomonas and nitrification has been examined. Studies completed by Rice and Pancholy (1972) and Montes and Christensen (1979) found a higher number of Nitrosomonas in early successional stages than in later successional stages, which corresponded with nitrate concentration. However, Robertson and Vitousek (1981) found the climax forest site they studied to have lowest numbers of Nitrosomonas and the higher nitrate production.

Environmental factors such as pH, soil type, temperature, oxygen supply, water regime, and vegetation also affect nitrification

(Alexander 1977; Barber 1984). The previous studies cited were performed on sandy soils with a minimum clay content. Sand has a low cation exchange capacity (CEC), which is the amount of exchangeable cations per unit of dry soil (Donahue et al 1983). It is measured in milliequivalents of cations per 100 grams of soil (meg/100 g). Cation nutrients, such as ammonium, do not move far through soil before they are adsorbed by exchange sites. Thus, sand, with a CEC of 1-5 would be less likely to retain ammonium than clay which has a CEC of over 30 (Donahue et al 1983). Barber (1986) examined nitrogen in a silty clay loam of eastern Kansas and found a dominance of ammonium, but considerable nitrate as well.

A comparison of nitrogen form between native prairie and an agricultural field of eastern Kansas was examined by Barber (1986). Although ammonium seemed to be dominant in the prairie, soil conditions were believed to be more important than vegetative cover. However, it has not yet been determined what nitrogen forms are present in reseeded old fields and if the relative amounts of ammonium and nitrate resembles those present in a climax prairie of similar soil type. These reseeded fields are usually planted with a mixture of five major grasses: Big Bluestem (Andropogon gerardi), Little Bluestem (Andropogon scoparius), Indian grass (Sorgashtrum nutans), Switchgrass (Panicum virgatum), and Side oats gramma (Bouteloua curtipendula) (all grasses are cited according to Hitchcock 1971). The purpose of this study was to determine the nitrogen form present in the reseeded fields and compare these with a climax prairie on the same or similar soil type.

The objectives were as follows:

1. to determine the form(s) of mineral nitrogen present in three different stages of reseeded fields and a native climax prairie, all on the same soil type, over the period of a year.
2. to study nitrification *in situ* on each of the study sites.
3. to record the numbers of *Nitrosomonas* present on each study site over the period of a year.
4. to examine total microbial activity on each site over the period of a year.

METHODS AND MATERIALS

Site Locations and Reseeding

Four sites, all located in Lyon County, Kansas, were selected for this study. The native pasture (site P) was located in the E 1/2 of the NW 1/4 of the NE 1/4 of section 27, Township 18S, Range 10E (Figure 1). Site S3 was an abandoned field which was reseeded in 1969 and was located in the N 1/2 of the SW 1/4 of the NW 1/4 of Section 3, Township 18S, Range 11E (Figure 2). Site S2 was an old field reseeded in 1981 and is adjacent to the pasture. It was located in the E 1/2 of the NE 1/4 of the NW 1/4 of Section 27, Township 18S, Range 10E (Figure 3). A severely eroded old field which had been reseeded in 1985 (site S1) was located in the E 1/2 of the SW 1/4 of the NE 1/4 of Section 17, Township 21S, Range 11E (Figure 4). All of the study sites were located on Kenoma soil series (Neill 1981). This is a silty clay loam with a one to three percent slope. Erosion had removed most of the original topsoil from the abandoned fields (Pritchard 1986).

Soil and Vegetation Properties

Information on the composition and amount of the seed mixture used on the reseeded areas was obtained from the Soil Conservation Service (Table 1) (Pritchard 1986). The soil pH was determined with a Beckman pH meter. Both active and reserve acidity were determined as described by Dahnke (1980). The hydrometer method of Bouyoucos (1936) was performed to determine soil texture. Vegetation on each site site was determined by the step loop method described by Wilk (1984). From this an average composition for the major species was determined.

Nitrate and Ammonium Determination

Soil samples were obtained from each site every three to four

Figure 1. Photograph of Site P.

Figure 2. Photograph of Site S3.

Figure 3. Photograph of Site S2.

Figure 4. Photograph of Site S1.

Table 1. Amount of pure live seed planted in pounds per acre.

Study Area	*Andropogon gerardi*	*Andropogon scoparius*	*Sorghastrum nutans*	*Panicum virgatum*	*Bouteloua curtipendula*
Site P - native prairie	---	---	---	---	---
Site S3	1.2	1.0	1.2	1.0	0.6
Site S2	1.1	1.1	1.2	0.5	1.1
Site S1	2.2	2.2	2.4	1.0	2.2

weeks for a year, beginning in January of 1986. Ten grams of the soil were placed in a 250 ml glass flask to which 100 ml of a 2 M potassium chloride (KCL) solution was added. The flask was then stoppered and placed on a mechanical shaker for one hour. The soil - KCL suspension was then filtered with Whatman No. 41 filter paper and the filtrate stored in a refrigerator until the sample could be analyzed.

The filtrate from each site was anaylzed for ammonium and nitrate by using the microdiffusion procedure described by Keeney and Nelson (1982). Each microdiffusion unit was divided into three chambers. One milliliter of boric acid indicator solution was added to the central chamber. To the peripheral sample chamber, two milliliters of the solution to be analyzed and one milliliter of 45 % potassium carbonate ($K_2 CO_3$) solution were added. Two milliliters of the 45 % K_2CO_3 were also added to the outer chamber to serve as a seal for the lid. Each microdiffusion unit was then placed in a constant temperature chamber at 25 C for 24 hours. During this period the potassium carbonate would liberate the ammonium present in the sample, which in turn was absorbed by the boric acid indicator solution in the central chamber. After 24 hours had passed the indicator solution was titrated with 0.02 N sulfuric acid solution to determine the amount of ammonium present.

The same samples were used to determine nitrate concentration. After sucking the central chamber clean, one milliliter of boric acid was added again. To liberate the nitrate, 0.05 gram of Devarda's Alloy was added to the sample chamber and the microdiffusion unit was placed in the constant temperature chamber for another 24 hour period. The nitrate was absorbed by the indicator solution and titrated as before. Tests for nitrite indicated none present in any of the samples. Both a blank and a standard solution with known amounts of ammonium and

nitrate were also tested.

The Most Probable Number of Nitrosomonas

The Most Probable Number (MPN) of Nitrosomonas present and a Total Plate Count (TPC) test were determined for each sample. This was done to examine the relationship between Nitrosomonas activity and the form of nitrogen. A dilution series to determine MPN and agar plates to provide a TPC were prepared as described by Schmidt and Belser (1982). Ten grams of soil from the sample were added to 95 milliliters of sterile, de-ionized water. The solution was mixed by shaking for ten minutes. Dilutions were made by mixing 10 milliliters of each dilution with 90 milliliters of sterile, de-ionized water. A series of ten dilutions was prepared for each sample. To prepare the Total Plate Count, one milliliter aliquots from each dilution were added to the center of sterile petri dishes. Enough general purpose medium was added to cover the bottom of the petri dish and the mixture was swirled. These plates were incubated at 25 C for one week and then the plates containing 30 to 300 organisms were counted.

To determine the Most Probable Number a one milliliter aliquot from each dilution series was transferred to each of five test tubes containing a sterile ammonium calcium carbonate solution. These tubes were incubated at 25 C for three weeks. After the incubation period the MPN of Nitrosomonas present was determined by adding three drops of modified Griess - Ilosvay reagent. If the solution turned pink the presence of Nitrosomonas was indicated. After determining the number from each dilution to turn pink, the MPN was calculated by using a table designed for ten - fold dilutions with five tubes per dilution (Alexander 1982).

A nitrification potential experiment designed by Barber (1986) was conducted in June and July. This involved removing a clump of grass and embedding a plastic soil ring approximately eight centimeters in diameter and five centimeters deep into the root zone. One hundred milliliters of 50 ppm ammonium sulfate was added to the soil in the ring and the clump of grass replaced over the ring. This was done with the purpose of disturbing the natural system as little as possible. A control ring to which no ammonium sulfate was added was also placed on each study site. After an incubation period of four weeks, the rings were collected and the soil was removed for analysis. The same tests were run on them as for the regular monthly samples.

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RESULTS AND DISCUSSION

Each of the study sites was located on the Kenoma soil series. This soil is an upland, moderately well drained soil that is slowly permeable (Neill 1981). An analysis of the chemical and physical characteristics from each site showed a clay content from 43 to 53 percent (Table 2). The pH determined in water varied from 5.4 at site S3 to 6.0 in the native prairie, while the pH in a 0.01 M calcium chloride was 4.4 to 4.5, thus suggesting a considerable amount of reserve acidity. A vegetative analysis of the dominant species found on each site is listed in Table 3. The dominant grass of the native prairie was Big Bluestem (Andropogon gerardi) while that of site S3 was Switchgrass (Panicum virgatum). Indian grass (Sorghastrum nutans) was the dominant grass on sites S2 and S1, however, a major species on S1 was three-awn (Aristida spp.). This would suggest S1 to be similar to an early pioneer stage in that respect, although the reseeding of the site is considered to be successful, and proper management should control the three-awn (Cavanaugh 1987). The native prairie and sites S3 and S2 were all burned in the spring of 1986. Each was grazed by cattle through the summer, with sites S2 and S3 exhibiting light to moderate use and the native prairie receiving quite heavy use.

Ammonium and Nitrate in the Soil

A minimum of four replicates from each study site were tested for ammonium and nitrate throughout the period of one year. The results of each one of these microdiffusion tests indicated there was less than 1.0 ppm of ammonium or nitrate, as the boric acid indicator solution showed virtually no color change (Table 4). Standards run with the replicates did release the predicted amounts of ammonium and nitrate. The minimum amount which could be detected with the available

Table 2. Chemical and physical characteristics of soil from each study site.

Characteristic	Prairie	S3	S2	S1
Soil Type	Silty Clay	Silty Clay	Silty Clay	Silty Clay
% Sand	7.0	5.0	3.0	5.0
% Silt	48.0	46.0	44.0	52.0
% Clay	45.0	49.0	53.0	43.0
pH [1]	6.0	5.4	5.5	5.5
pH [2]	4.4	4.4	4.5	4.5

1 pH determined in water

2 pH determined in 0.01 M calcium chloride

Table 4. Concentrations of ammonium and nitrate (in ppm) in soil from native prairie and reseeded old fields. Each value is a mean of at least four replicates.

Date	Prairie		S3		S2		S1	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
01-21-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
02-22-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
03-22-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
04-20-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
05-28-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
06-28-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
07-25-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
08-26-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
09-27-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
10-28-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
11-22-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
12-19-86	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

equipment was approximately 1.0 ppm or somewhat less, as indicated by the standard curve (Figure 5). However, to verify the near absence of these forms of nitrogen, a set of samples was sent to the Kansas State University soils lab for analysis. The Technecon Nitrogen Analyzer there detected less than one half of a part per million on any of the samples (Table 5). These were the same samples in which titrations showed less than 1.0 ppm in the lab at Emporia State University. To further verify that such a low amount of mineral nitrogen was present, actual soil samples were sent to the Cooperative Extension Service Soil Testing laboratory at Kansas State University. Test results from these samples indicated the nitrogen level on all sites to be less than 1.0 ppm. It was noted that even in such small amounts ammonium was the dominant nitrogen form. This agrees with the results recorded by Barber (1986) in a recent study of a native pasture on a similar soil series, although the amounts were much less.

The Most Probable Number (MPN) of Nitrosomonas and a Total Plate Count (TPC) to determine the number of all soil microbes present were taken for each sample date. Only one reading for each site was determined, due to the long incubation time for these tests and the limited amount of equipment available. The overall mean number of Nitrosomonas for the year was lowest for the native prairie and highest for site S1, the most recently reseeded area (Table 6). Although these numbers are quite variable due to environmental conditions of the season, they agree with the results of previous researchers (Rice and Pancholy 1972; Montes and Christensen 1979) who also found the lowest number of Nitrosomonas in the climax areas studied. Overall numbers of the total plate counts (Table 7) were lowest in the prairie and highest for site S1. The magnitude of the numbers involved are such that the difference

Figure 5. Standard curve for titration to determine ammonium and nitrate concentration, using 0.02 N sulfuric acid.

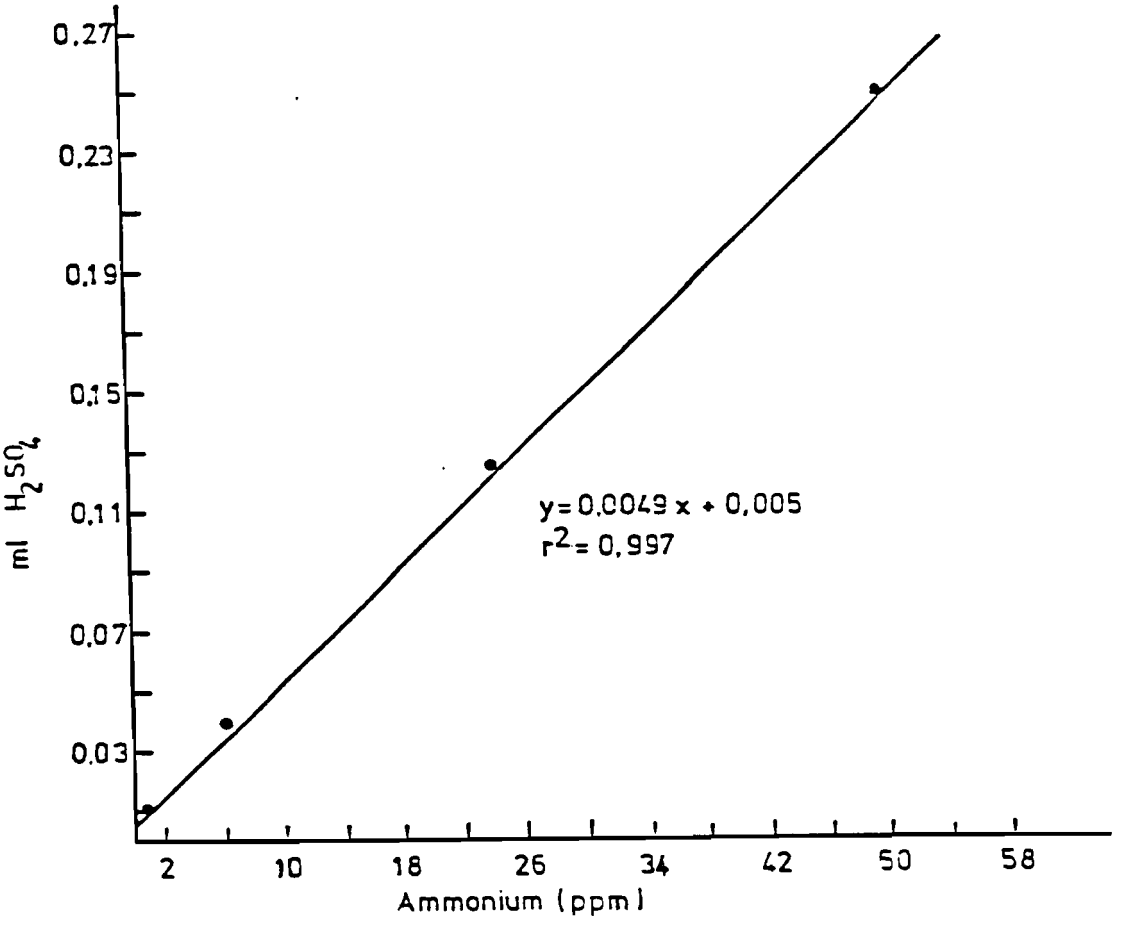

0.27
0.23
0.19
0.15
0.11
0.07
0.03
ml H_2SO_4
y= 0.0049 x + 0.005
r^2= 0.997
2
10
18
26
34
42
50
58
Ammonium (ppm)

Table 5. Concentrations of ammonium and nitrate (in ppm) from native prairie and reseeded fields. Samples analyzed by two laboratories.

	Prairie		S3		Site 2		Site 1	
Laboratory	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Kansas State University	0.35	0.0	0.25	0.06	0.31	0.02	0.21	0.0
Emporia State University	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Analysis of the same samples by two different labs.

Table 6. Most Probable Number of Nitrosomonas (organisms/gram soil) for native prairie and reseeded fields.

Date	Pasture	S3	S2	S1
01-27-86	2.0	170.0	490.0	110.0
02-22-86	0.0	49.0	790.0	22.0
03-22-86	110.0	490.0	780.0	49.0
04-20-86	0.0	33.0	1300.0	330.0
05-28-86	11.0	17.0	330.0	4900.0
06-28-86	20.0	130.0	170.0	70.0
07-25-86	130.0	490.0	330.0	1100.0
08-26-86	9.2	170.0	1300.0	490.0
09-27-86	20.0	490.0	700.0	230.0
10-28-86	230.0	1300.0	330.0	1300.0
11-22-86	4.0	6.8	170.0	170.0
12-19-86	0.0	4.0	27.0	79.0
Mean #	44.7	279.2	559.8	737.5

Table 7. Total Plate Counts (x 10^6) for native prairie and reseeded fields.

Date	Pasture	S3	S2	S1
01-27-86	8.0	8.1	TNTC*	TNTC*
02-22-86	10.0	9.1	14.7	8.75
03-22-86	9.6	11.2	12.4	12.1
04-20-86	11.9	7.8	13.2	45.0
05-28-86	8.1	6.2	6.2	15.4
06-28-86	10.4	4.7	8.1	12.6
07-25-86	10.0	8.9	11.3	9.5
08-26-86	13.6	20.0	11.6	41.0
09-27-86	15.3	44.0	30.0	29.0
10-28-86	10.3	36.0	13.0	29.0
11-22-86	11.2	7.9	27.0	15.9
12-19-86	7.6	9.7	5.8	9.8
Mean #	10.5	14.5	13.9	20.7

* Too numerous to count

between study sites is really small and the total numbers do not differ greatly from those determined by Barber (1986).

Nitrification Potential

A nitrification potential study was conducted using a technique developed by Barber (1986). Whereas previous researchers (Robertson and Vitousek 1981; Lamb 1980) conducted such experiments by taking the soil samples to a laboratory, this method involves nitrification *in situ*. A clump of soil with vegetation intact was removed and a soil ring was placed in the root zone. Fifty ppm of ammonium as ammonium sulfate were added to each ring. The vegetation and soil clump were carefully replaced, causing as little disturbance as possible. After a four week incubation period, soil from each ring was collected and the microdiffusion test, Most Probable Number of *Nitrosomonas*, and Total Plate Counts were determined.

On April 17, 1986, 10 ml of fifty ppm ammonium sulfate were added to soil rings on the native prairie and site S2 and left until May 12, 1986. Microdiffusion tests indicated the ammonium and nitrate present to be less than 1.0 ppm. The MPN of *Nitrosomonas* in the native prairie showed an increase, with 130 organisms per gram of soil compared to 0 and 11 organisms per gram of soil found in the April and May samples. A slight increase in the Total Plate Count of 8.1×10^6 to 14.2×10^6 was noted. Site S2 showed a MPN increase of 330 to 700 organisms per gram of soil and TPC increase of 6.2×10^6 to 8.5×10^6.

Since little ammonium or nitrate was detected in the microdiffusion tests, another experiment was set up in which 100 ml of 50 ppm ammonium as ammonium sulfate was added to each soil ring. This application of ammonium was made at each study site on June 12, 1986,

and July 16, 1986. The soil samples collected approximately four weeks later contained a very large amount of ammonium and much less nitrate (Table 8).

The Most Probable Number of Nitrosomonas determined after the addition of ammonium sulfate (Table 9) showed an increase for both months in the native prairie and site S1. Reseeded sites S3 and S2 remained approximately the same in July, while S3 increased in August and S2 decreased. Total Plate Counts varied little in July (Table 10) but increased in August on every study site except S3. These results do not support Lamb's (1980) proposal of limited availability of ammonium being the cause of nitrification inhibition.

The high levels of ammonium present could have been due to the breakdown of organic matter due to the addition of the ammonium sulfate. The addition of inorganic nitrogen can promote mineralization, probably due to the response of the general microbial community (Alexander 1977). However, even with the increased ammonium the numbers of Nitrosomonas did not greatly increase and the amount of nitrate produced was minimal compared to the amount of ammonium. Previous studies (Munro 1966; Neal 1969; Rice and Pancholy 1973, 1974; Rice 1984) have suggested that the climax prairie vegetation inhibits nitrification. In examining the mean number of Nitrosomonas over the period of a year (Table 6), there does seem to be a trend for the numbers of Nitrosomonas to decrease from the most recently reseeded area to the native pasture. Although the nitrification potential experiments did show some increase in nitrate and the numbers of Nitrosomonas, the amount was fairly small. These results suggest that the climax grasses seeded in the old fields are affecting the nitrogen

Table 8. Ammonium and nitrate present (in ppm) after the addition of 50 ppm ammonium as ammonium sulfate to native prairie and reseeded fields.

		June				July	
Study Site		NH_4^+	NO_3^-	Study Site		NH_4^+	NO_3^-
Pasture	A	246.0	11.5	Pasture	A	114.0	6.0
	B	227.0	11.5		B	53.0	6.0
	C	206.0	11.5		C	116.0	4.0
	D	191.0	11.5		D	*	*
	E	*	*		E	*	*
	Mean	217.5	11.5		Mean	94.3	5.3
	Control	< 1.0	< 1.0		Control	< 1.0	< 1.0
S3	A	90.5	4.0	S3	A	107.0	4.6
	B	70.0	4.0		B	116.0	4.0
	C	146.0	6.0		C	196.0	4.0
	D	74.0	6.0		D	106.0	4.0
	E	131.0	4.0		E	125.0	4.0
	Mean #	102.3	4.8		Mean	130.0	4.0
	Control	< 1.0	< 1.0		Control	< 1.0	< 1.0
S2	A	159.0	6.0	S2	A	135.0	4.0
	B	162.0	6.0		B	81.0	4.0
	C	171.0	6.0		C	142.0	4.0
	D	182.0	8.0		D	142.0	4.0
	E	159.0	6.0		E	111.0	4.0
	Mean #	166.6	6.4		Mean	122.2	4.0
	Control	< 1.0	< 1.0		Control	< 1.0	< 1.0
S1	A	164.0	8.0	S1	A	107.0	8.0
	B	122.0	6.0		B	132.0	6.0
	C	125.0	6.0		C	92.0	6.0
	D	138.0	8.0		D	77.0	4.0
	E	*	*		E	95.0	6.0
	Mean	137.3	7.0		Mean	100.6	6.0
	Control	< 1.0	< 1.0		Control	< 1.0	< 1.0

* Soil ring pulled out before sample taken.

Table 9. Most Probable Number of Nitrosomonas after adding 50 ppm ammonium as ammonium sulfate (organism/gram soil) for native prairie and reseeded fields.

Date	Pasture	S3	S2	S1
Treated 07-18-86	790.0	490.0	330.0	1300.0
Untreated 07-25-86	130.0	490.0	330.0	1100.0
Treated 08-05-86	1100.0	790.0	330.0	790.0
Untreated 08-26-86	9.2	170.0	1300.0	490.0

Table 10. Total plate count after adding 50 ppm ammonium as ammonium sulfate (x 10^6) for native prairie and reseeded fields.

Date	Pasture	S3	S2	S1
Treated 07-18-86	9.5	10.0	9.0	8.7
Untreated 07-25-86	10.0	8.9	11.3	9.5
Treated 08-05-86	55.0	13.0	79.0	49.0
Untreated 08-26-86	13.6	20.0	11.6	41.0

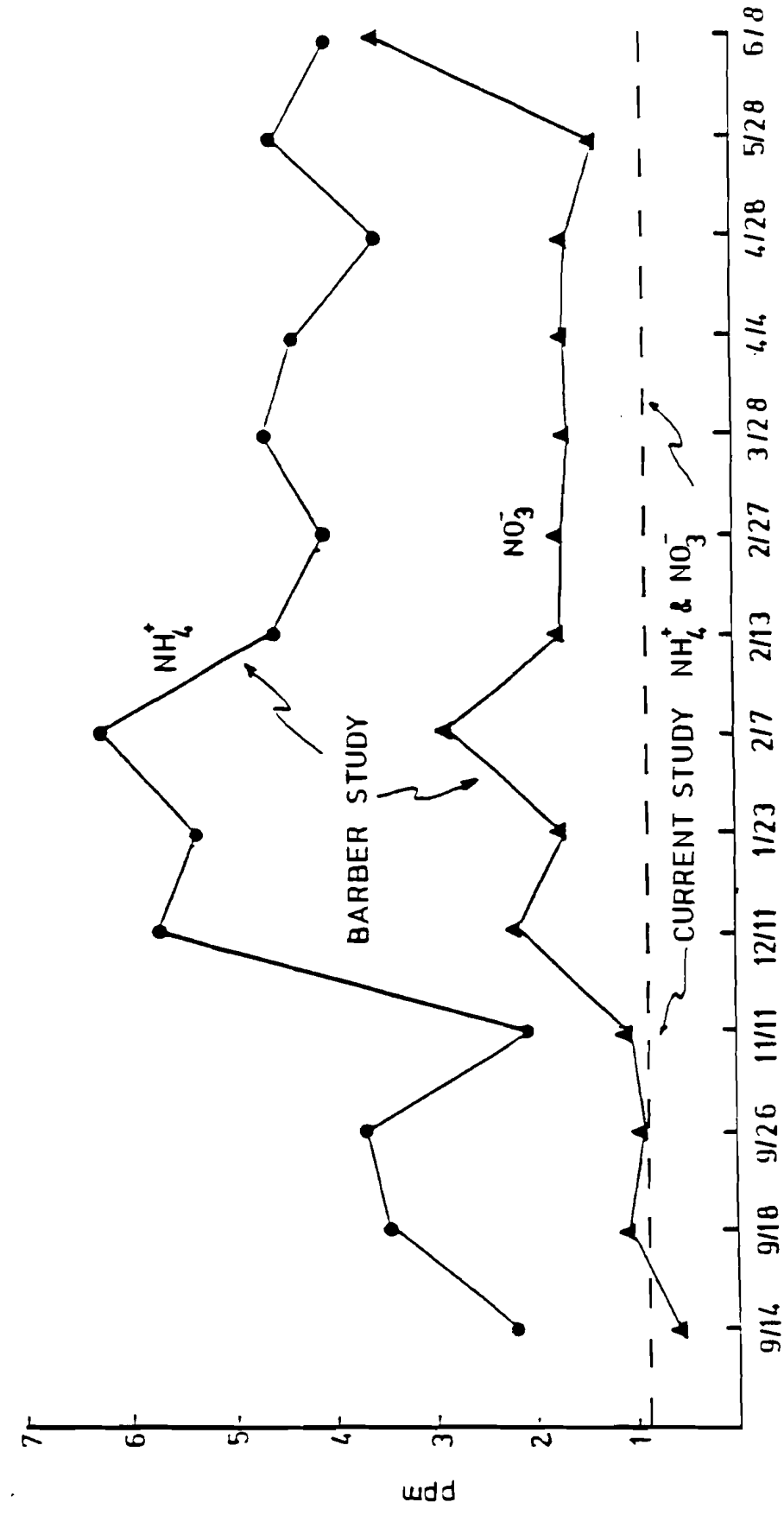
forms present there in much the same manner as the climax prairie. This agrees with the result of Rice and Pancholy (1972; 1973; 1974) and Rice (1984) which propose that it is the climax vegetation which inhibits nitirification.

The results obtained on nitrogen forms present were unexpected. It was anticipated the ammonium level would be lowest in the area reseeded in 1985 and highest in the native prairie; and that nitrate would be lowest in the native prairie. However, these data indicate that nitrogen may be a limiting nutrient for the plants on all of the study sites. This was particularly unexpected on the native prairie, since the study on a similar native prairie of similar soil type (Barber 1986) indicated both ammonium and nitrate to be present in greater amounts (Figure 6). Thus, other factors which could be causing this difference were examined.

Other Factors

In May of 1986, the overall range condition of the native prairie was determined to be good, using the condition class analysis of Wilk (1984). Cattle were allowed to graze there throughout the summer and fall, with use appearing to be quite heavy. The native prairie studied by Barber (1986) experienced no grazing, but had been hayed the previous year. A comparison of biomass production was made using ten 0.25 square meter plots on each prairie site. The vegetation was clipped, dried, and weighed to compare production. The prairie site of this study had a biomass production of 323.84 grams per square meter (2889.23 pounds per acre) while the prairie of Barber's (1986) study had 434.72 grams per square meter (3878.48 pounds per acre). This was a statisticaly significant difference, at the 0.05 level, of 110.88 grams per square meter (989.25 pounds per acre). This difference in

Figure 6. Comparison of ammonium and nitrate concentrations in ppm of Barber's study (1986) and the native prairie from this study.


ppm
7
6
5
4
3
2
1
NH_4^+
NO_3^-
BARBER STUDY
CURRENT STUDY NH_4^+ & NO_3^-
9/14
9/18
9/26
11/11
12/11
1/23
2/7
2/13
2/27
3/28
4/4
4/28
5/28
6/8
DATE

production could be due to the greater nitrogen supply available at Barber's prairie site.

Continual removal of vegetation can also promote a loss of nitrogen. In perennial plants, after the seed demand for nitrogen has been met, the nitrogen moves to the crown and roots to be available for the next seasons growth (Salisbury and Ross 1978). With heavy grazing weakening the crown of the plant, less nitrogen will be available for the next seasons growth or returned to the soil. A study of the revegetation of abandoned fields in Kansas and Oklahoma indicated heavy pasturing was not beneficial to plant succession and could also cause these areas to remain unproductive longer than they otherwise would (Booth 1941). Thus, the heavy grazing on the pasture site could be influencing the nitrogen supply and productivity. This could also apply to study sites S3 and S2 which were light to moderately grazed.

Study site S2 was located adjacent to the native prairie with no fence dividing the two areas. During the summer and fall it was noted that the cattle seemed to prefer the native prairie to the reseeded area. A range utilization evaluation based on the stubble height method (Wilk 1984) showed Little Bluestem (Andropogon scoparius) utilization to be 71 % on the native prairie and only 13 % on the reseeded area. This indicates that utilization of the native prairie was quite heavy, whereas the reseeded area received very light use (Figure 7). This preference for native prairie over reseeded areas by cattle has been previously noted, though not documented, by ranchers in the area. The question arises as to why this preference exists. Perhaps the native prairie is more palatable while the seed used to revegetate the abandoned fields has been developed for seed production

Figure 7. Photograph of range utilization difference between Site P, foreground, and Site S2, background.

rather than palatability. Another possible explanation could be that the less fertile soils of the old fields produce a poor quality, less-palatable forage. The nitrification potential studies suggest that although the amount of nitrate and the number of Nitrosomonas increase after the addition of ammonium, these increases are slight. This may indicate that all of the study sites are behaving somewhat like a climax prairie and supressing nitrification. The increase in the amount of ammonium present over that which was added suggests an increase in the mineralization of organic nitrogen. This increase cannot be attributed to simple disturbance, as the controls showed no increase in either ammonium or nitrate. These are questions which deserve further investigation.

The results of this study indicate the native prairie and the reseeded old fields which were examined to have very low amounts of ammonium and nitrate present. The low nitrate level is further reflected by the low Nitrosomonas levels. Overall microbial activity determined by the Total Plate Counts showed little variation between study sites and the numbers were similar to those obtained by Barber (1986).

The comparison of overall productivity of the native prairie site and the prairie examined by Barber (1986) showed productivity to be much greater at her site. It was further noted that the levels of ammonium and nitrate, and the number of Nitrosomonas were greater in her native prairie. This difference in results was possibly related to the heavy grazing which occurred on the control prairie site. The low levels of ammonium and nitrate, and the small population of Nitrosomonas on all study sites (although a trend in reseeded areas was

noted) suggests that the climax prairie grasses are the important components affecting the nitrogen forms present. Further studies examining the effects of grazing on the nitrogen pool and the overall productivity would be beneficial.

SUMMARY

A study of the ammonium and nitrate present in a native prairie and reseeded old fields was conducted from January 1986 through December 1986. All of the study sites were located on the Kenoma soil series. Monthly samples were taken from each site and analyzed using the microdiffusion procedure of Keeney and Nelson (1982). The Most Probable Number of Nitrosomonas and Total Plate Count were also determined for each sample using a dilution series as described by Schmidt and Belser (1982). A nitrification potential experiment was set up in situ to minimize disturbance to the soil and vegetation.

Results from the microdiffusion tests indicated less than 1.0 ppm of ammonium or nitrate to be present in all of the study sites throughout the year. The Most Probable Number of Nitrosomonas was also low on all sites, though a decreasing trend from the most recently reseeded site to the native prairie (738 to 45) was noted. After the addition of 50 ppm ammonium as ammonium sulfate, the number of Nitrosomonas and the amount of nitrate increased only slightly. A great increase in ammonium was detected, possibly due to the mineralization of organic nitrogen. Overall production was much lower in the control prairie site than that of a similar prairie which had not been heavily grazed. This suggests that the use of the prairie as a pasture could be affecting productivity and the nitrogen pool present. However, the low level of ammonium and nitrate present and the small population of Nitrosomonas on all sites suggest the climax prairie grasses are affecting the nitrogen forms present.

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