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SOIL NITROGEN INVESTIGATIONS ON THE PAWNEE SITE, 1970

J. O. Reuss and P. W. Copley

Agronomy Department

Colorado State University

GRASSLAND BIOME

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ABSTRACT

Nitrogen fixation studies were conducted by an acetylene reduction technique on a variety of soil cores from the Pawnee Site under varying conditions of aeration, temperature, and energy supply. Both highly artificial, soluble energy source and anerobic conditions are required to achieve significant free living fixation. At saturation without artificial energy source, a maximum of a few g/ha/day are fixed. At field moisture, fixation is less than 1 g/ha/day. Under otherwise favorable conditions, a temperature regime of 0°C during the night and 16°C during the day essentially stopped fixation; and, when temperatures were increased, fixation was much less than in soils not subjected to cold treatment. Fixation rates on single potted legume plants were highly reproducible. No N fixation could be detected in pond waters.

Sampling variability studies indicate a high variability in total N content between similar soil mapping units at different locations. Where fertilizer N was spring applied at the rate of 150 kg/ha, an average of 60 kg/ha remained as mineral N in the top 40 cm of soil. A few measurements of N in precipitation averaged about 1 ppm N.

INTRODUCTION

In 1970 the major effort was again centered on rates of biological nitrogen fixation at the Pawnee site. These rates were investigated by the acetylene reduction technique as described in the 1969 report (Reuss and Copley 1969), but with considerable refinement of technique. In addition, some data were collected in each of the following areas: (i) variations of total nitrogen levels within and between mapping units, (ii) mineral nitrogen levels in fertilized and unfertilized plots, and (iii) nitrogen precipitation. Part I of this report is concerned with the N fixation investigations, and the other studies are included in Part II.

PART I. NITROGEN FIXATION INVESTIGATIONS

Experimental Methods

The basic method of evaluating N fixation involves the evaluation of nitrogenase activity by determining the rate of reduction of acetylene to ethylene. The method is widely used for determining rates of fixation of legume nodules, mocrobial cultures, cell free extract, etc., but has previously been used only to a very limited extent on undisturbed soil cores or soil-plant systems. The method was described in part in a previous report (Reuss and Copley 1969), but techniques were improved substantially during the 1970 season. A brief description follows with emphasis on the 1970 changes.

Measurements on undisturbed soil cores were made on cores 8.12 cm in diameter and 12 cm deep. Plants grown in the greenhouse or growth chamber were grown in pots 8.5 cm in diameter and filled to a depth of 13 cm. The complete core, or pot and plant, was placed in a plexiglass fixation chamber for the determination. The atmosphere in the chamber was replaced with He or an 80:20 He:0_2 mixture containing 5% C_2H_2. The C_2H_4 produced by reduction of the C_2H_2 by the nitrogenase enzyme system was determined by gas chromatography. Test runs with standard concentrations of C_2H_2 demonstrated that the concentration was not affected by the presence of the soil, i.e., the ethylene was not adsorbed on the soil material. Time of acetylation varied, depending on the rates of fixation, but for legume systems was generally five to six hours and for nonsymbiotic systems from 100 to 250 hours. In those systems where respiration was likely to exceed photosynthesis, excess CO_2 was removed by placing a small vial of 2N KOH in the chamber. The fixation chambers were placed in a small growth chamber so that light and temperature could be regulated.

The fixation chambers were connected to a source of 0_2 through a small, specially constructed mercury check valve. As the 0_2 in the chamber was depleted by soil respiration, the CO_2 evolved was obsorbed in the alkaline trap. This caused a slight pressure decrease which resulted in the automatic replacement of 0_2 through the mercury check valve. The system worked well and generally maintained 0_2 levels within $\pm$ 5% during runs that sometimes extended over several days.

Acetylene reduction techniques were also used in attempts to detect N fixation in temporary ponds and the permanent pond (Cottonwood) on the site. These involved both laboratory incubations of the pond waters and incubation in vials in the pont itself. Methods used were essentially those of Howard et al. (1970).

All calculations reported have been converted to N fixed on an area basis and have been made on the assumption that three moles of C_2H_2 produced in the system is equivalent to the fixation of one mole of N_2. Rates are determined by plotting N fixation as a function of time and determining the slope of the linear portion of the curve. At low levels of fixation, the method can easily detect rates as low as 1 g/ha/day. Duplicate cores often exhibit substantial variation and ranges of 30% of the mean between duplicates are not uncommon for the higher rates. Where fixation rates are very low, ranges may be several hundred percent of the mean even though absolute variation is low.

Results and Discussion

Experiment 1. The effect of a moisture level below field capacity, as well as two rates and two sources of energy at field capacity moisture, are shown in Table 1. The systems were all aerobic, utilizing the 80:20 He:O_2 atmosphere. These were run at a room temperature of approximately 21°C, and the rates were obtained by plotting the data shown in Appendix Table 1. In several cases there was an unusually long lag period of 30 hours or more before measurable fixation started. These determinations were made before the automatic oxygen replacement system was developed, resulting in more "noise" in the data than in some of the later work. Some of the points at the longest times are inconsistent, probably due to excessive changes in oxygen level during the run. In these cases the earlier, more linear portions of the curve were used in determining the rate. In the relatively dry system (5% moisture) and at field capacity without an added energy source, nitrogen fixation was virtually nonexistent. At the field capacity moisture level with rates of added sugar in the range of 280 to 560 kg C/ha,

Table 1. The effect of moisture and energy levels on rates of nonsymbiotic nitrogen fixation in undisturbed upland cores of Ascalon sandy loam under aerobic conditions.

Moisture Level	Approx. % H_2O	Energy Source	Rate of Sugar	N_2 Fixed		
				A	B	$\bar{y}$
			kg C/ha	g/ha/day		
Field Moisture	5	None	-	0.1	0.3	0.2
Field Capacity	18	None	-	0.3	0.1	0.2
Field Capacity	18	Sucrose	280	1.6	0.6	1.6
Field Capacity	19	Sucrose	560	3.1	10.0	6.5
Field Capacity	19	Glucose	280	0.8	12.0	6.5
Field Capacity	19	Glucose	560	19.0	2.0	10.5

rates of fixation varied from less than 1 g/ha/day to a maximum of 19 g/ha/day. The individual cores were quite variable in this range, and this variability was sufficient to mask any possible small differences in rates or sources of energy material. Even the highest rate, achieved in the presence of a highly exaggerated energy supply, would only represent the fixation of 1.9 kg/ha/100 days.

Experiment 2. Table 2 summarizes the results of an experiment conducted to evaluate the effect of rates of sucrose added to saturated upland and bottomland cores from the Pawnee Site. Fig. 1 and 2 show the plots of the actual data for the 280 kg C/ha rates. On the bottomland soil the rates and total N fixed by the duplicate cores were very similar and are shown by a single line. The duplicates were somewhat different on the upland cores, so both were plotted. Actually, the rates of the duplicates are very similar, but one core exhibited a longer lag phase and a lower total fixation. There seems to be a rather general pattern that longer lag phases are associated with lower total fixation. Most likely the energy source is being utilized by both fixing and nonfixing organisms, and a longer lag phase allows more to be used by the nonfixers. The plots for the other rates and soils are shown in Appendix Fig. 1 and 3.

Table 2 definitely indicates that on the bottomland soils the higher fixation rates are associated with the higher rates of sucrose. Due to missing data the pattern cannot be detected for sure on the upland soil. These data were lost due to the leakage of the chambers during the run. It is interesting to note that for both soils and all rates of sugar the total N fixed was about 10 g per kg carbon added. The reason that no data are given in Table 2 for total fixation in the upland soil at the 560 kg C/ha

Table 2. Nitrogen fixation on saturated upland and bottomland cores at different rates of added sucrose.

Source	Carbon Added	Maximum Fixation Rate	N Fixed Per Kg Carbon
	kg/ha	kg/ha/day	g
Upland	140	No data	-
	280	2.0	9
	560	2.4	No data
Bottomland	140	0.6	9
	280	1.7	10
	560	3.4	9

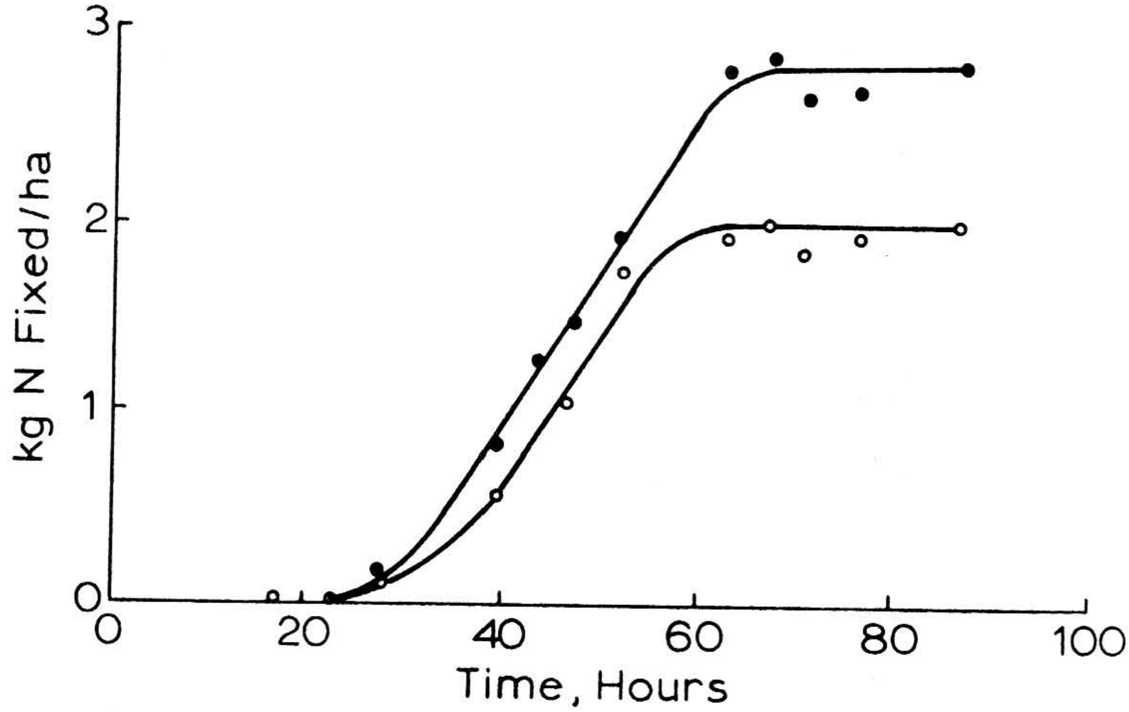

Fig. 1. Nitrogen fixation of duplicate cores of upland soil under saturated moisture conditions and with the addition of sucrose at the rate of 280 kg C/ha.

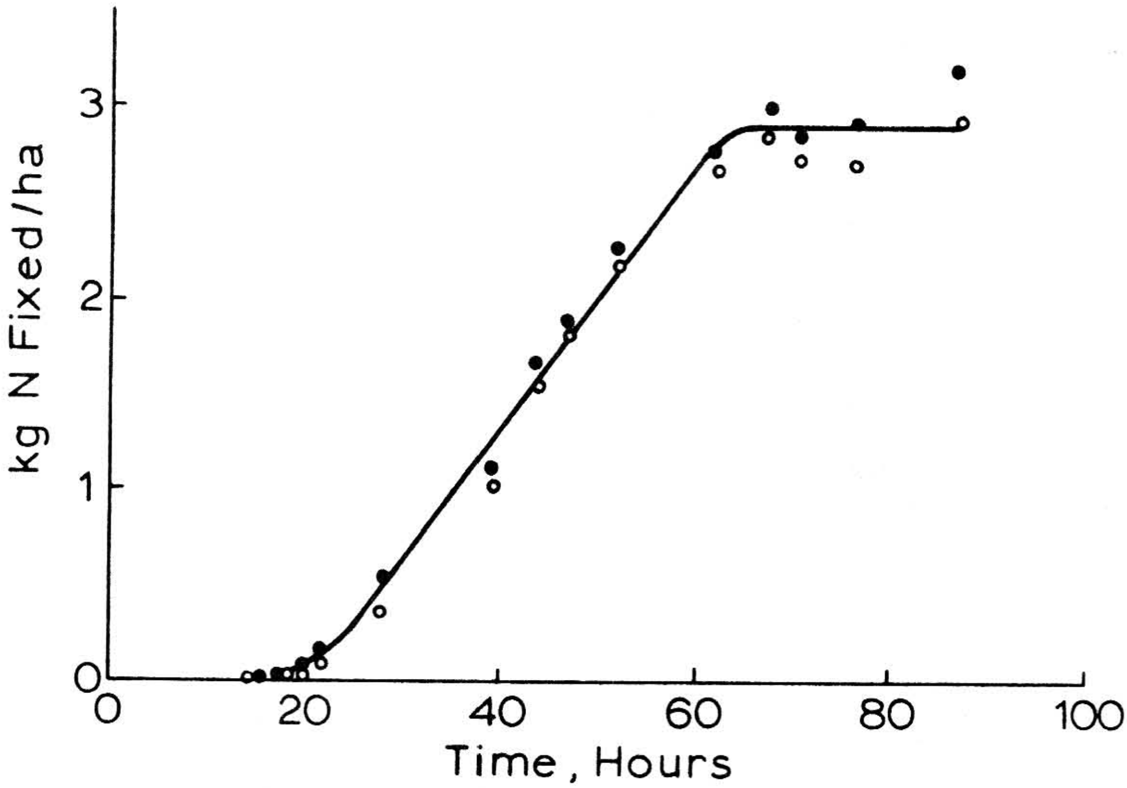

Fig. 2. Nitrogen fixation of duplicate cores of bottomland soil under saturated moisture conditions and with the addition of sucrose at the rate of 280 kg C/ha.

is that the fixation had not completely stopped in these cores at the end of the run. Note that very high rates of fixation were achieved under these conditions, particularly on the bottomland soil where the highest rate of carbon added resulted in 3.4 kg N/ha/day being fixed.

The atmosphere over these soils was maintained at about the normal 20% oxygen, but the soils were kept in a saturated moisture condition and relatively high rates of a very soluble energy source were added. Previous experience (Reuss and Copley 1969) indicates that under saturated moisture conditions without additional energy sources, rates are generally under 10 g/ha/day. Thus, high fixation rates can be achieved but apparently require both saturated soil conditions and a source of readily available energy.

The relatively low total fixation of 10 g N/kg C is important. Thus, if 600 kg dry matter containing 50% C were decomposed under saturated conditions, we would only expect the fixation of 3 kg nitrogen. Actually, most decomposition probably takes place at moisture levels at or below field capacity. The low fixation resulting from the addition of sugar at these moisture levels in Table 1 indicates that at lower moisture most decomposition is carried out by nonfixing organisms.

Experiment 3. The results of an experiment designed to investigate possible temperature effects on fixation are summarized in Table 3. The original intention was to conduct the experiment at 0°C, but it was found that the growth chamber would not maintain this temperature under full light conditions. The actual temperature regime attained was 12 hours of light at 16°C followed by 12 hours of darkness at 0°C. Under this treatment only one core attained a rate above 1 g/ha/day and the average was only 0.4. For all practical purposes fixation was nonexistent even though the addition

Table 3. Nitrogen fixation rates in saturated soil cores held at 0°C dark and 16°C light for 138 hours followed by 120 hours at 27°C.

Description	16°C Light, 0°C Dark			27°C		
	138 Hours			130 Hours		
	A	B	$\bar{y}$	A	B	$\bar{y}$
	g/ha/day			g/ha/day		
1. Disturbed upland soil, + sugar[a/]	0.6	0.1	0.4	204	105	154
2. Temporary lake bottom, no sugar	0.4	0.8	0.6	9	22	16
3. Temporary lake bottom, - sugar	1.8	0.9	1.4	102	64	84
4. *Buchloe dactyloides* - sugar	0.4	0.4	0.4	78	25	52
5. *Agropyron cristatum*, + sugar	0.4	0.1	0.3	34	13	24
6. *Artemisia frigida*, - sugar	0.5	0.5	0.5	57	26	42
Mean undisturbed uplands, with sugar			0.4			39

[a/] Sugar treatment was the equivalent of 326 kg C/ha applied as sucrose.

of 326 kg C/ha as sucrose and saturated moisture conditions provided otherwise satisfactory conditions for fixation.

After 138 hours the temperature was raised to 27°C. Fixation did start to take place, but at a much lower rate than would be expected for this temperature with saturated moisture and with sugar added. There was considerable variation in duplicate cores under these conditions, but six saturated upland cores with 280 kg C/ha added averaged only 39 g N fixed/ha/day as compared to 2.0 kg/ha/day with 280 kg C/ha as shown in Table 2 for upland soils on which no cold pretreatment was imposed. Thus, Table 3 indicates that the reduced temperatures not only stopped fixation but reduced subsequent fixation rates after warming. One interesting aspect of Table 3 is the results after warming for the lake bottom soils where no energy source was added. The fixation rates were higher than in most previous experiments without added sugar, but still only averaged 16 g/ha/day. The data gathered are included in Appendix Tables 3 and 4. Rates reported in Table 3 are obtained from graphs plotted from this data.

Experiment 4. The data in Table 4 summarize the results from an experiment conducted to check the results of a preliminary experiment reported in 1969. This was designed to determine whether the lack of fixation in unsaturated soils was due to the aerobic conditions or whether saturated moisture conditions per se are necessary. The data are also presented in Fig. 3 in graphic form. Appendix Tables 5 and 6 show the actual data that were plotted.

Here again, fixation was negligible at field capacity moisture under an atmosphere containing 20% O_2, even though sugar was added to all treatments. Fixation was highest in saturated soils under a helium atmosphere.

Table 4. Mean fixation rate as affected by moisture status and atmospheric composition on disturbed upland cores of Ascalon sandy loam (326 kg C/ha added).

Description	Atmosphere	Fixation Rate kg N/ha/day	Efficiency g N/kg C
Aerobic, saturated	80:20-He:0_2	1.2	10.1
Anerobic, saturated	He	2.5	15.5
Aerobic, field capacity	80:20-He:0_2	0.0013	-
Anerobic, field capacity	He	1.2	5.2

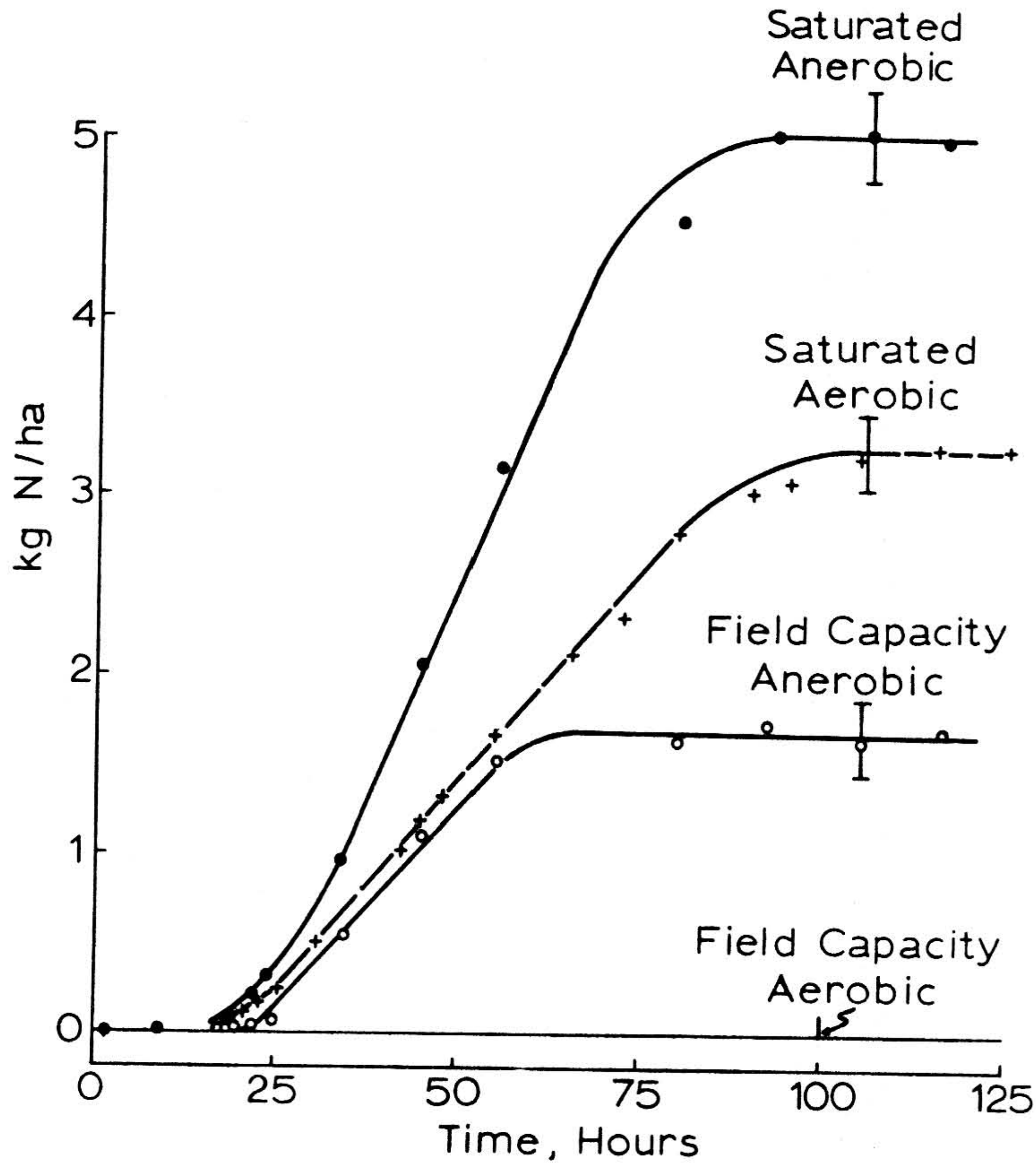

Fig. 3. Mean N fixation as affected by moisture status and atmospheric composition on disturbed cores of Ascalon sandy loam amended with 326 kg C/ha as sucrose. The vertical bars represent ±2(S.E. of the mean) at that time.

Fixation rates were similar under a helium atmosphere at field capacity and under a He:O_2 atmosphere with saturated moisture conditions, but the saturated aerobic treatment produced more total fixation than the helium atmosphere at field capacity. Apparently, the organisms are largely aerobic and will fix N at less than saturation moisture levels. However, even under artificially anerobic conditions, they will fix more rapidly at saturation moisture levels than at field capacity, and the total N fixed per unit of carbon is greater at saturation.

Statistical analysis of such data is plagued with problems of homogeneity of variances, but a reasonable evaluation of the variation can be attained by considering the standard error of the mean at any point. The vertical bars on Fig. 3 at the 115 hour time indicate $\pm$ 2($SE_{\bar{y}}$) of the individual cores at that time. These indicate that there is a high probability the differences are due to the treatments imposed.

Experiment 5. Fig. 4 to 6 show the results of fixation determinations on single legume plants grown in the greenhouse.

Single 30-day-old potted plants of *Medicago sativa* L. (alfalfa) and *Onobrychis viciaefolia* Scop. (sainfoin) were placed in the fixation chambers. The rates are expressed on an area basis as calculated from a 56.75 cm^2 pot. Plants were 30 days old at the time of the run. Note that for both species the duplicate plants gave almost identical rates. The scales are quite different for Fig. 4 and 5, but excellent straight line relationships were obtained in both cases. The two lines are plotted on the same scale in Fig. 6. The single 30-day old alfalfa plants were fixing N at a rate of 1.09 kg/ha/day while the sainfoin rate was 0.156 kg/ha/day. Previous attempts to measure rates of plants removed from the field had met with only limited

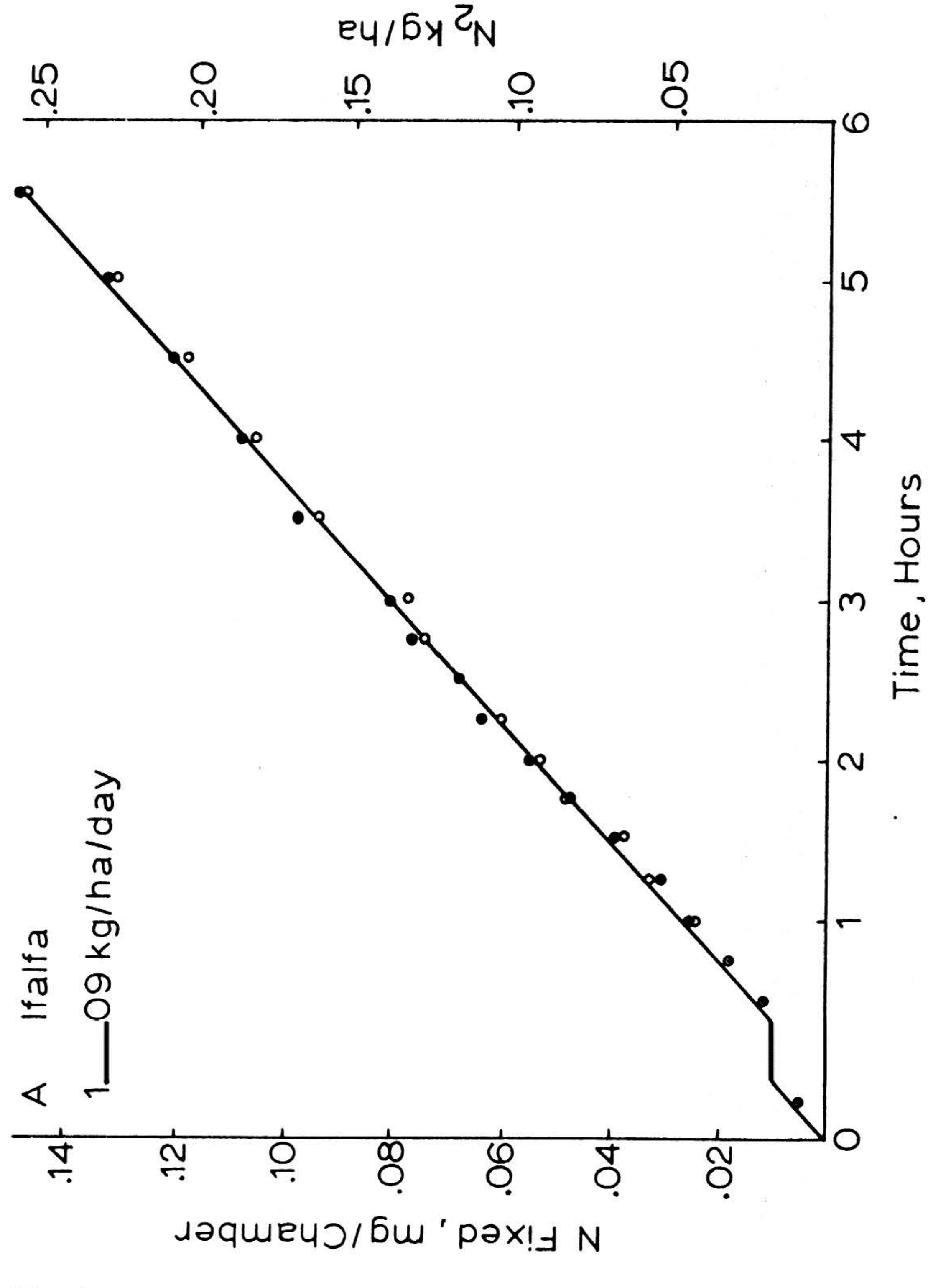

Fig. 4. Nitrogen fixation by duplicate 30-day-old single potted plants of *Medicago sativa* L. (alfalfa).

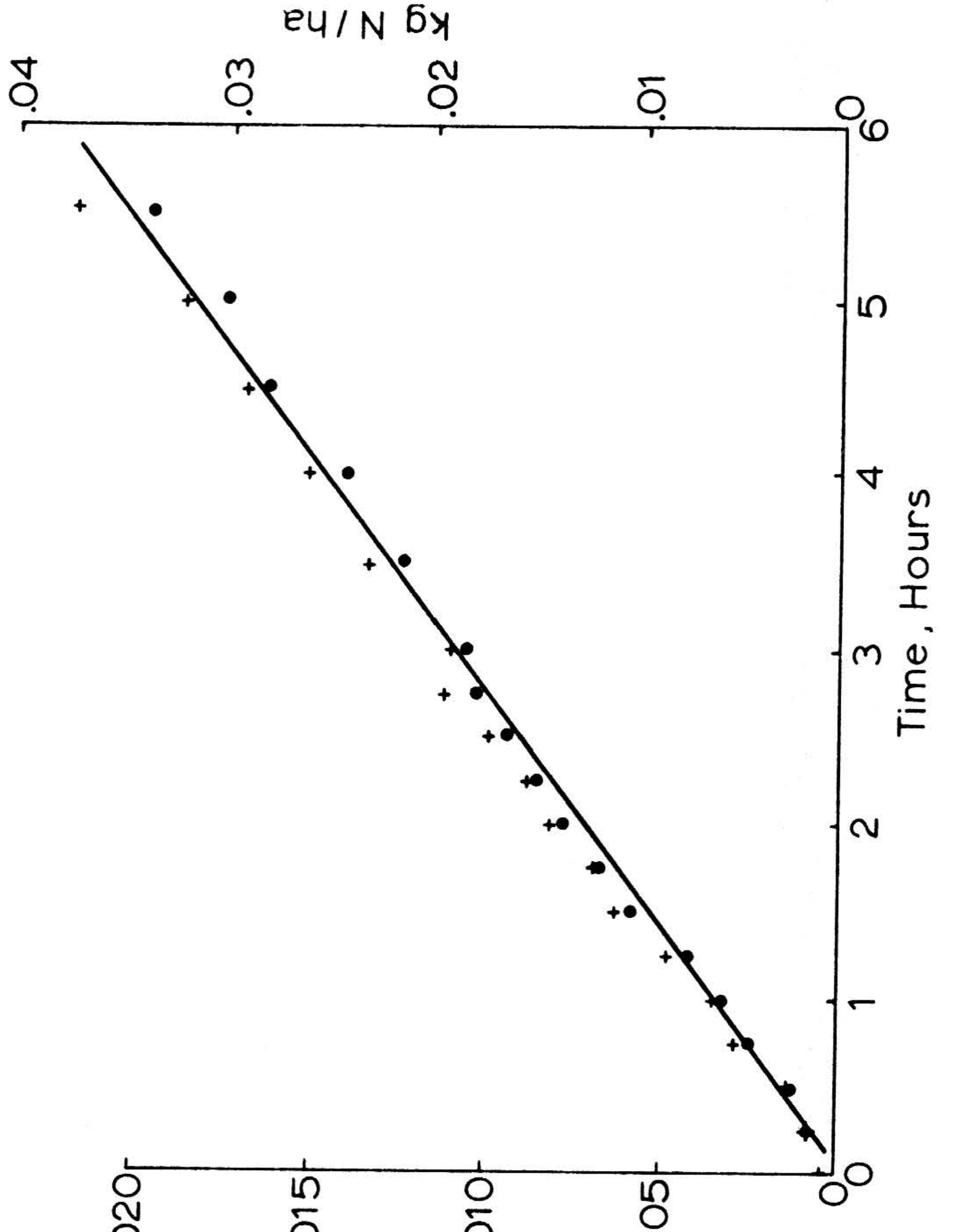

Fig. 5. Nitrogen fixation by duplicate 30-day-old single potted plants of *Onobrychis viciaefolia* Scop. (sainfoin).

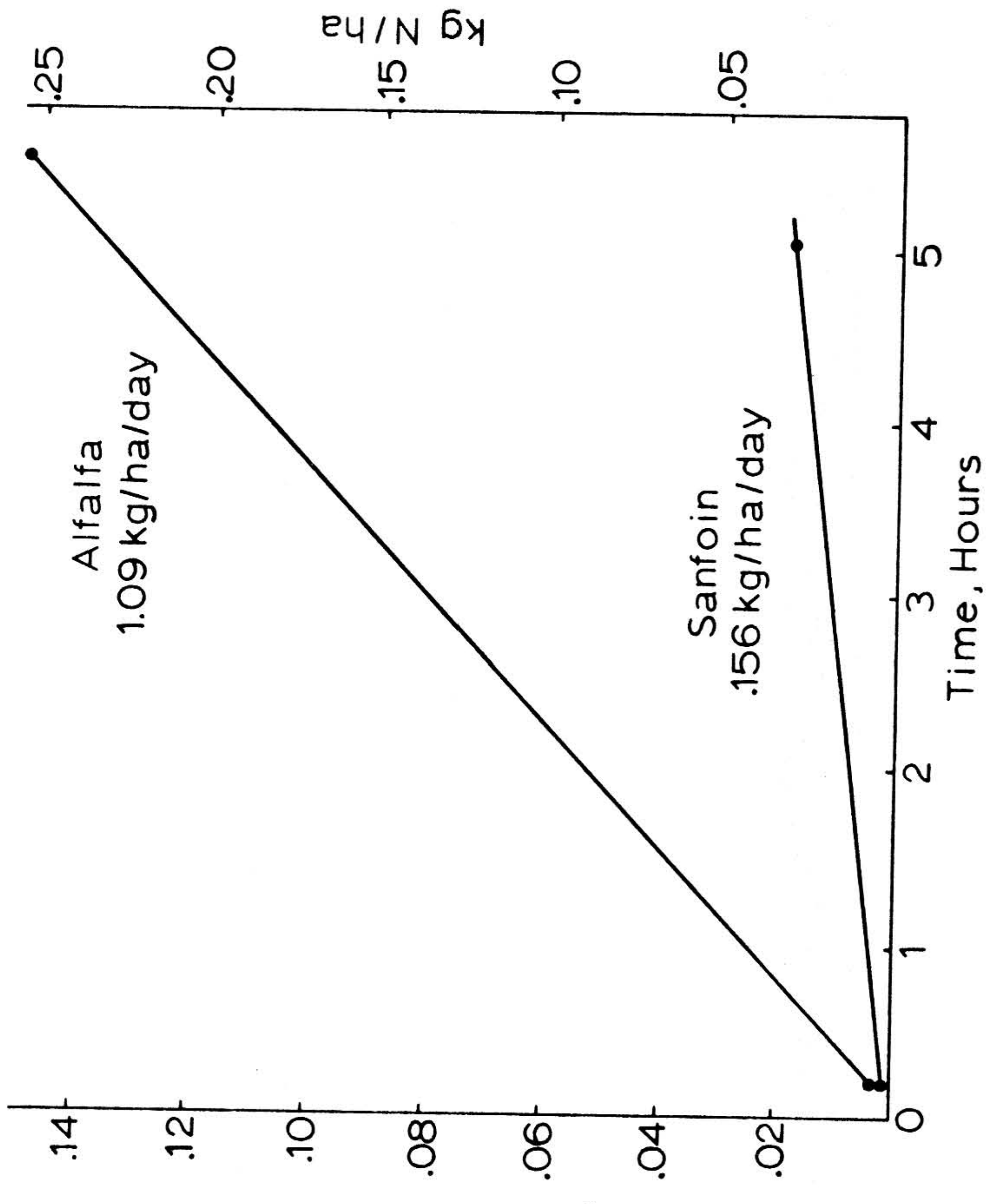

Fig. 6. Comparison of nitrogen fixation rates by single potted plants of *Medicago sativa* L. (alfalfa) and *Onobrychis viciaefolia* Scop. (sainfoin).

success. The excellent results obtained with these plants is very encouraging, and more extensive measurements involving both additional species and ages of plants are presently underway.

All attempts to detect nitrogen fixation in the waters of either temporary ponds or in the permanent Cottonwood pond were unsuccessful. These attempts involved acetylation, both in the laboratory and in vials placed in the ponds. Apparently no fixation was taking place, at least during the July to September period, when these tests were conducted. The data were all negative and are not included in the report.

PART II. MISCELLANEOUS NITROGEN STUDIES

Experimental Methods

Total N sampling variation study. Two locations were selected, each containing areas of selected mapping units. The mapping units were: (i) 55 AB-Ascalon sandy loam 0-4% slope and (ii) 55 C-Ascalon sandy loam 5-8% slope. Location 1 included parts of the W½ Sec 21 and W½ Sec 28, while location 2 involved parts of the E½ Sec 22 and E½ Sec 27. Using the standard soil survey, a contiguous area of each mapping unit was selected in each location. Ten sampling points were then randomly selected in each area. A schematic diagram of the sampling scheme is shown in Appendix Fig. 4. A sample consisting of 10 cores from the 0 to 10 cm depth was taken at each sampling point. These samples were air dried, ground to pass a 30-mesh sieve, and analyzed in duplicate for total organic nitrogen using a micro-Kjeldahl procedure.

Mineral nitrogen measurements on fertilizer nitrogen experiment. An experiment was established in the spring of 1970 to evaluate the effect of nitrogen fertilizer and irrigation treatments on the Pawnee Site. Briefly, eight 1 ha plots were established, consisting of two replications of two nitrogen treatments (none and 150 kg/ha), each applied with and without supplemental irrigation. The mineral nitrogen analysis was the only phase of this experiment conducted as part of the nitrogen investigations, so only those results are reported here.

Samples for mineral nitrogen analysis were collected in mid-October. Sampling depths and numbers of cores per plots on the various treatments are shown in Table 5. The lack of samples below 40 cm and the low number of cores per plot below 20 cm on the nonirrigated treatments was due to the practical difficulties involved in sampling the dry soils at that time of year. The variable number of cores below 40 cm on the irrigated plots was due to the fact that depth of moisture penetration varied slightly, and collection of samples below the depth of moisture penetration proved to be impractical. Separate samples were taken from the north and south half of each plot.

Samples were air dried and passed through a 2 mm sieve prior to analysis. They were then extracted with 2N KCl and NH_4^+, determined on the extract distillation with MgO, and titrated with standard HCl. Devardas alloy was then added to reduce the NO_3^- and the distillation and titration repeated.

Determination of nitrogen in precipitation. A few precipitation and runoff samples were collected by the Hydrology Group late in the season. The precipitation samples were collected in plastic rain gages. The runoff samples were collected by a sample collecting devise at the measuring

Table 5. Sampling scheme for mineral nitrogen analyses on irrigation-fertilization experiment.

Irrigated		Non-irrigated	
Depth cm	No. Cores Per Sample	Depth cm	No. Cores Per Sample
0-10	10	0-10	10
10-20	10	10-20	10
20-40	10	20-40	5
40-60	Variable		
>60	Variable		

flumes on the micro-watershed. The samples were stored in frozen condition until the analyses could be run. The NH_4^+ and NO_3^- were determined by the same distillation methods that were used on the soil extracts for mineral N determinations.

Results and Discussion

Mean total N contents for the locations and mapping units are shown in Table 6. As shown in the analysis of variance in Table 7, there was much more variation between mapping units within locations than between either locations or units. Thus, it would probably not be practical to try to determine the effect of treatments applied at different locations on the total N content of mapping units within those locations unless such treatments could be expected to have produced large differences. There was quite a large variation between samples taken within a mapping unit, but by taking 10 samples a satisfactory estimate of the total N content can be attained. This is indicated by the $SE_{\bar{y}}$ of 29 ppm within units shown in Table 8. The $SE_{\bar{y}}$ of a single determination on a given sample of 22 ppm indicates satisfactory precision in the analytical determinations. The results of the individual analyses are shown in Appendix Table 7.

Mineral Nitrogen Experiment

A summary of the mineral nitrogen analyses from the fertilizer-irrigation experiment is shown in Table 9, and the individual data are included in Appendix Table 8. The fertilized plots had an average of 60 kg N/ha more mineral N in the 0 to 40 cm depths than the nonfertilized plots. This represents 40% of the N applied in the spring. Unfortunately, variation was high and even this large mean difference due to fertilizer

Table 6. Summary of mean total soil N in the 0-10 cm depth in each of two mapping units found at two locations.

Locations	Mapping Unit 55AB ppm N	55C ppm N	$\bar{y}$ ppm N
1	750	685	717
2	735	830	784
$\bar{y}$	742	758	750

Table 7. Analysis of variance on duplicate total N analyses from each of two mapping units at two locations.

Source	DF	SS	MS	F
Total	79	828,449		
Locations	1	83,076	83,076	
Units	1	4,500	4,500	
LXU	1	127,680	127,680	7.74**
Samples	36	593,829	16,495	35.10**
Determinations	40	19,365	484	

** Significant at 1% level.

Table 8. Selected standard errors of total N analyses of 0-10 cm depth.

Source	No. of Samples in Mean	No. of Determinations in Mean	Standard Error
			ppm
Between Units and Locations[a/] (Estimate any similar unit and location by sampling one unit and location)	10	20	60
Within Units (Estimate mean of a unit and location by sampling that unit and location)	10	20	29
Between Samples within Units (Estimate mean of unit with one sample)	1	2	91
Determinations within Samples (Estimate sample from duplicate determinations)	1	2	16
Single Determinations	1	1	22

a/ Pooled mean square.

Table 9. Means and standard errors for nitrate plus ammonium nitrogen found from 0-40 cm as affected by fertilization and irrigation.

	Assumes Bulk Density of 1.25		
	Fertilized[a/]	Not Fertilized	Mean
	kg/ha	kg/ha	kg/ha
Irrigated	96	33	65
Not irrigated	91	34	63
Mean*	94	34	63.8

Standard Errors of the Mean

Source	Obs. in Mean	$SE\bar{y}$
Irrigation	8	9.0
Fertilizer	8	9.0
FXI	4	12.6
Samples within plots	2	12.0

Standard Errors of a Single Observation

Source	$SE\bar{y}$
Plots within reps and treatments	25.4
Samples within plots	17.0

a/ 150 kg N/ha applied as ammonium nitrate.

* Significant at 5% level.

treatments was only significant at the 5% probability level. The variation between plots within reps and treatments was higher than that between samples within plots. Even so, the sample variation is undesirably high, and sampling methods should be modified in the future.

It is of interest to note that 34 kg/ha of mineral N were found in the top 40 cm. Most estimates of nitrogen cycled to the aboveground portions of the plant in this system are in the range of 10 to 15 kg/ha/year. Thus, the NH_4^+ and NO_3^- nitrogen in the top 40 cm represents about two to three times the amount cycled to the aboveground portions in an average season. Only about 8.5 kg/ha are in the nitrate form, but even this represents more than one-half that cycled to the aboveground portion annually. It must be recognized, however, that this only represents an average of 1.6 ppm N as NO_3^- and at such small contents analytical errors could be significant.

Rainfall and Runoff Determinations

The analyses of the rainfall and runoff samples collected by the Hydrology Group in September are shown in Appendix Tables 9 and 10. While these data only represent a small proportion of the year's events, a general order-of-magnitude estimate can be calculated for precipitation inputs. Thus, a weighted mean indicates about 1 ppm mineral nitrogen in the precipitation over this period. Extrapolating to a yearly 300 mm precipitation on this basis would suggest an input of 3 kg N/ha/year. The runoff seems to be insignificant in the two events measured. While an arithmetic mean is not strictly valid in this case, it does indicate a very low mineral nitrogen loss. The runoff from watershed 1 can be estimated as 0.4 g and that from watershed 3 as 0.04 g mineral N/ha. These low values result from the very low fraction of the precipitation measured as runoff.

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APPENDIX I

APPENDIX TABLES

Appendix Table 1. Nitrogen fixation on upland disturbed cores under aerobic conditions at various energy and moisture levels. Values are calculated as g N_2/ha.

Time (Hrs)	Field Moisture No Energy		Field Capacity No Energy		Field Capacity 280 kg C/ha Sucrose		Field Capacity 560 kg C/ha Sucrose		Field Capacity 280 kg C/ha Glucose		Field Capacity 560 kg C/ha Glucose	
	A	B	A	B	A	B	A	B	A	B	A	B
0.5	.50	.50	1.25	1.25	1.25		1.25					1.25
10.0	.50	.70	.75	1.10	.50	1.10	.75	1.10	1.25	1.25	1.35	1.00
14.5	.75	1.40	1.10	1.25	1.50	1.50	1.25	1.35	1.25	1.45	1.30	1.20
17.0						1.45		1.50	1.60			1.20
23.5	1.25	1.30	1.20	1.25	2.60	1.60	2.25	2.00	1.60	1.70	1.70	1.40
34.0	1.15	1.40	1.30	1.20	2.90	1.80	3.30	3.00	1.80	1.85	1.85	1.25
47.0					4.15	1.95	3.65	5.65	2.35	4.80	3.70	1.55
65.0	1.60	1.65	1.65	1.85	6.25	2.65	7.80	17.30	3.50	16.00	18.40	3.95
82.0					6.00	2.60	8.20	22.40	3.50	20.80	26.60	5.30
89.0	1.45	1.50	1.45	1.70	6.10	2.80	7.50	24.00	3.15	26.00	34.00	6.00
111.0	1.40	1.50	1.50	1.35	6.60	3.40	8.75	35.00	4.10	35.00	55.00	7.25
138.0	1.35	1.65	1.65	1.95	7.30	3.65	8.95	51.00	5.55	51.00	87.00	8.45
160.0	1.35	2.05	2.80	3.05	11.05	5.40	13.80	93.00	10.00	73.00	145.00	14.40

Pertinent information:

Soil type - Ascalon sandy loam.
Room temperature.
Atmosphere - 80:20 He:O_2 + 5% C_2H_2.
Cores - 8.12 cm diameter × 12 cm deep.

Appendix Table 2. Moisture levels determined on cores used in experiment reported in Appendix Table 1. (Percent)

Depth (cm)	Field Moisture No Energy		Field Capacity No Energy		Field Capacity 280 kg C/ha Sucrose		Field Capacity 560 kg C/ha Sucrose		Field Capacity 280 kg C/ha Glucose		Field Capacity 560 kg C/ha Glucose	
	A	B	A	B	A	B	A	B	A	B	A	B
0-4	2.9	3.1	19.5	20.9	20.6	19.3	22.8	22.2	22.4	23.6	22.1	20.4
4-8	4.1	6.1	17.7	18.6	17.0	18.4	18.1	17.1	17.4	17.6	19.4	17.6
8-12	7.4	9.0	17.9	16.3	16.9	17.4	16.8	16.7	17.0	16.0	19.2	17.5
Mean	4.8	6.1	18.3	18.6	18.1	18.3	19.2	18.6	18.9	19.0	20.2	18.5

Appendix Table 3. Nitrogen fixation in six saturated soil cores held at 0°C dark and 16°C light for 138 hours followed by 120 hours at 27°C. Values are g/ha.

Time (Hours)	Temperature	Disturbed Bare Soils With Sugar		Temporary Lake Soils			
				No Sugar		With Sugar	
		A	B	A	B	A	B
2.5	0-16°C	1.8	1.8	1.8	1.4	2.1	1.8
14.0	0-16°C	1.4	1.8	1.8	2.1	2.4	2.4
24.0	0-16°C	1.8	1.8	2.1	2.4	2.4	2.8
127.0	0-16°C	2.4	4.2	2.8	5.6	10.1	6.3
147.0	27°C	41.0	39.0	5.6	17.5	57.0	39.0
169.0	27°C	234.0	143.0	10.1	35.0	143.0	83.0
193.0	27°C	526,0	372.0	19.2	72.0	258.0	132.0
252.0	27°C	974.0	622.0	46.0	115.0	339.0	179.0

Appendix Table 4. Nitrogen fixation in six saturated soil cores held at 0°C dark and 16°C light for 138 hours followed by 120 hours at 27°C. Values are g/ha.

Time (Hours)	Temperature	*Buchloe dactyloides* + Sugar		*Agropyron cristatum* + Sugar		*Artemisia frigida* + Sugar	
		A	B	A	B	A	B
13	0-16°C	2.5	1.8	1.8	1.8	2.8	2.8
18	0-16°C	1.8	2.1	2.1	1.4	2.8	3.1
25	0-16°C	2.8	2.8	2.1	2.1	3.1	3.5
36	0-16°C	2.8	2.1	2.4	2.1	2.8	3.1
138	0-16°C	4.2	4.2	3.8	2.4	4.2	4.2
158	27°C	10.1	10.5	8.4	6.3	10.1	11.6
180	27°C	39.0	31.0	17.0	17.0	44.0	58.0
204	27°C	125.0	69.0	53.0	48.0	94.0	181.0
261	27°C	305.0	112.0	131.0	67.0	115.0	260.0

Appendix Table 5. Nitrogen fixed in disturbed cores of Ascalon sandy loam under a 80:20 He:O_2 atmosphere at saturation and field capacity moisture levels. 326 kg C/ha as sucrose added to all treatments. Values are g N/ha.

Time (Hours)	Field Capacity				Saturated			
	A	B	C	$\bar{y}$	A	B	C	$\bar{y}$
2.0	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
18.5	2.6	2.6	2.2	2.5	46	64	61	57
19.5					74	102	88	88
21.0			2.2		107	144	117	123
23.0	3.2	3.2	3.2	3.2	166	223	173	187
26.0			3.2		254	343	253	283
31.0	3.9	3.9	4.2	4.0	471	621	450	514
42.0	3.9	3.9	5.1	4.3	1085	1228	880	1064
45.0					1291	1382	994	1222
48.0	4.5	4.8	5.8	5.0	1451	1525	1085	1354
55.0	4.5	4.8	6.4	5.2	1896	1902	1325	1708
66.0	4.5	4.8	7.1	5.5	2485	2433	1582	2167
72.0	4.8	4.8	7.4	5.7	2707	2559	1748	2338
79.0	5.1	5.1	8.7	6.3	3336	3084	2039	2820
90.0	6.4	6.1	9.0	7.2	3633	3370	2233	3079
95.0	6.4	6.1	9.0	7.2	3667	3450	2330	3149
104.0	6.4	6.1	9.3	7.3	3838	3519	2490	3282
115.0	6.7	6.4	9.3	7.5	3838	3553	2559	3312
125.0	7.1	6.4	10.0	7.8	3930	3667	2650	3315
162.0					3838	3587	2445	3290

Appendix Table 6. Nitrogen fixed in cores of Ascalon sandy loam under a helium atmosphere at saturation and field capacity moisture levels. 326 kg C/ha as sucrose added to all treatments. Values are g N/ha.

Time (Hours)	Field Capacity				Saturated		
	A	B	C	$\bar{y}$	A	B	$\bar{y}$
2.0	1.6	1.6	1.9	1.7	2.1	2.1	2.1
9.0	2.2	2.2	2.2	2.2	2.8	3.2	3.0
19.5	4.8	5.5	3.2	4.5	108	82	95
22.5	30	46	13	30	222	185	204
24.5	69	108	49	75	353	276	314
34.0	489	678	549	572	1099	860	980
45.0	1001	1263	1099	1121	2335	1823	2079
55.0	1417	1705	1438	1517	3554	2814	3184
79.0	1674	1900	1479	1684	5012	4169	4590
92.0	1771	2003	1510	1761	5582	4557	5070
105.0	1685	1890	1459	1678	5514	4523	5018
116.0	1736	1952	1500	1729	5501	4580	5040

Appendix Table 7. Duplicate total N determinations from 10 sampling points in each of two mapping units at two locations.

Sampling Point	Location 1				Location 2			
	55AB		55C		55AB		55C	
	ppm N		ppm N		ppm N		ppm N	
1	671	685	736	761	651	674	648	648
2	694	702	692	696	727	769	875	906
3	722	792	694	692	683	662	826	807
4	713	743	641	699	747	766	764	777
5	721	734	621	602	725	712	758	792
6	636	640	624	612	644	627	978	958
7	735	759	782	857	750	743	899	907
8	915	1027	613	707	794	835	903	919
9	833	824	532	523	826	791	890	889
10	671	688	807	814	784	794	685	767

Appendix Table 8. Ammonium and nitrate nitrogen levels by depth, nitrogen fertilization, and irrigation treatments.

Treatment	Plot No.	Rep.	Depth	South NH_4^+	South NO_3^-	North NH_4^+	North NO_3^-
			cm	ppm N		ppm N	
Non-fertilized Non-irrigated	3	I	0-10	3.7	1.5	3.7	0.0
			10-20	2.6	1.8	4.4	0.0
			20-40	7.0	0.4	3.7	0.0
Non-fertilized Non-irrigated	5	II	0-10	7.7	4.2	7.2	2.4
			10-20	7.2	3.7	7.1	2.4
			20-40	7.7	1.8	4.0	0.4
Non-fertilized Irrigated	2	I	0-10	4.3	1.7	7.3	0.3
			10-20	2.3	1.0	7.3	3.7
			20-40	4.4	2.4	2.2	0.0
			40-60	3.4	0.9	1.5	1.3
			>60			2.6	0.0
Non-fertilized Irrigated	9	II	0-10	3.8	3.4	9.6	6.8
			10-20	3.0	3.3	3.9	3.2
			20-40	3.3	0.0	7.7	1.5
			40-60	6.1	2.2	12.1	6.2
Fertilized[a/] Non-irrigated	7	I	0-10	25.2	5.5	42.8	9.9
			10-20	2.4	2.4	3.7	6.2
			20-40	8.8	2.6	6.6	0.0
Fertilized Non-irrigated	10	II	0-10	42.3	10.1	17.5	8.4
			10-20	3.3	27.4	3.0	32.3
			20-40	4.8	0.0	3.2	0.9
Fertilized Irrigated	8	I	0-10	14.3	7.0	6.6	0.4
			10-20	3.3	9.0	5.0	2.2
			20-40	5.7	5.5	4.2	11.0
			40-60	4.0	3.1	5.3	6.1
Fertilizer Irrigated	4	II	0-10	33.3	10.3	24.3	8.0
			10-20	8.3	11.3	5.7	6.7
			20-40	6.3	12.7	7.7	12.5

a/ kg N/ha applied as NH_4NO_3.

Appendix Table 9. Mineral nitrogen measured in precipitation.

Date	Micro Watershed	Precipitation[a/]	NH_4^+	NO_3^-	Sum
		mm	ppm N	ppm N	ppm N
9/2/70	1-8	4.8	1.14	0.11	1.25
9/12-9/14/70	1-8	4.0	0.92	0.77	1.69
9/16/70	1-8	1.0	0.38	1.50	1.85
9/22/70	1, 2, 3	5.1	0.26	0.15	0.41
	4, 5	12.2	0.22	0.43	0.65
	6, 7, 8	12.3	1.34	0.04	1.38
9/24/70	1-8	5.8	0.37	0.13	0.50
			Weighted Mean		0.98

a/ Precipitation reported is arithmetic mean of those gauges reporting measurable precipitation.

Appendix Table 10. Mineral nitrogen measured in runoff.

Date	Micro Watershed	Sample	NH_4^+	NO_3^-	Sum	Runoff
						mm
9/2/70	1	11	1.05	0.33	1.38	
	1	12	1.19	1.19	2.38	
				Mean	1.88	0.02
	3	2	0.48	0.53	1.01	
		3	0.26	0.22	0.48	
		4	0.28	0.11	0.39	
		5	0.39	0.10	0.49	
		6	0.15	0.07	0.22	
		7	0.22	0.13	0.35	
		8	0.04	0.09	0.13	
		9	0.26	0.13	0.39	
				Mean	0.43	0.01

APPENDIX II

APPENDIX FIGURES

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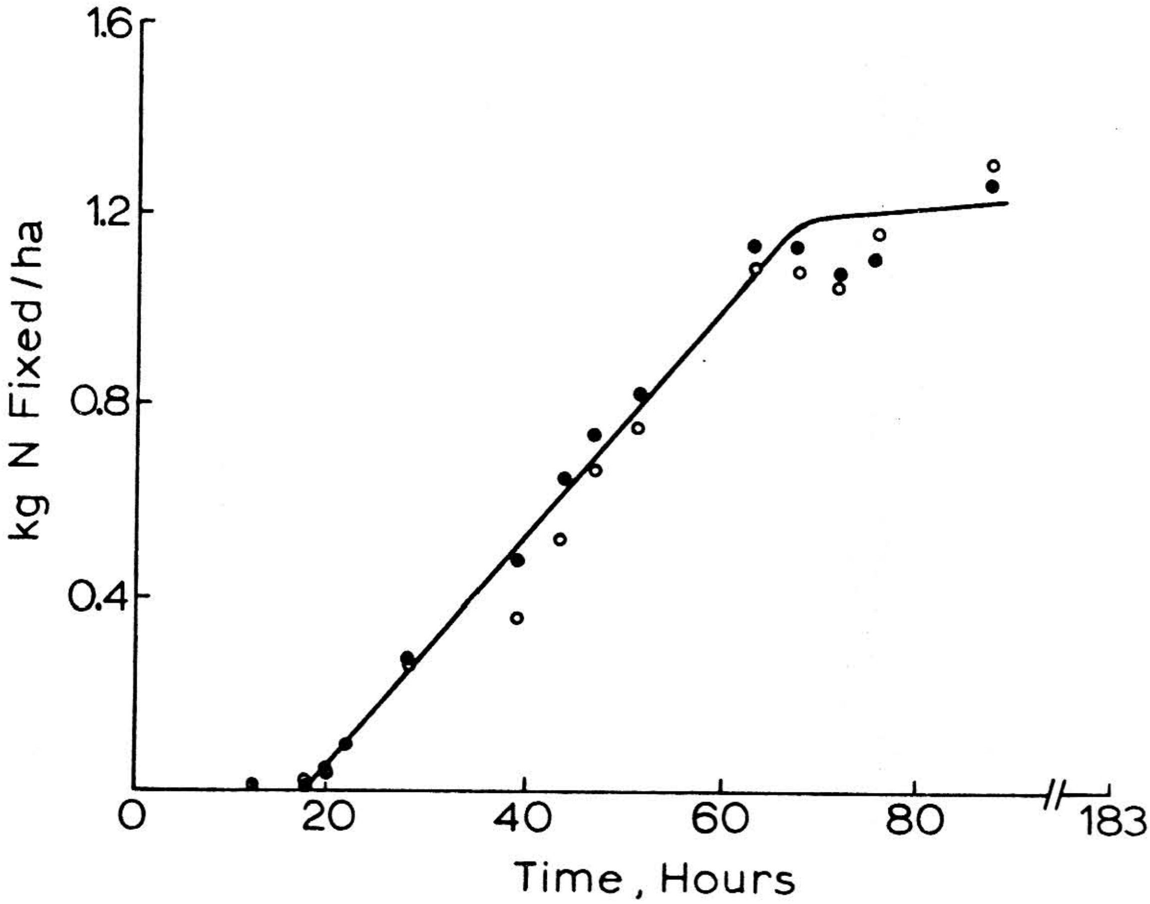
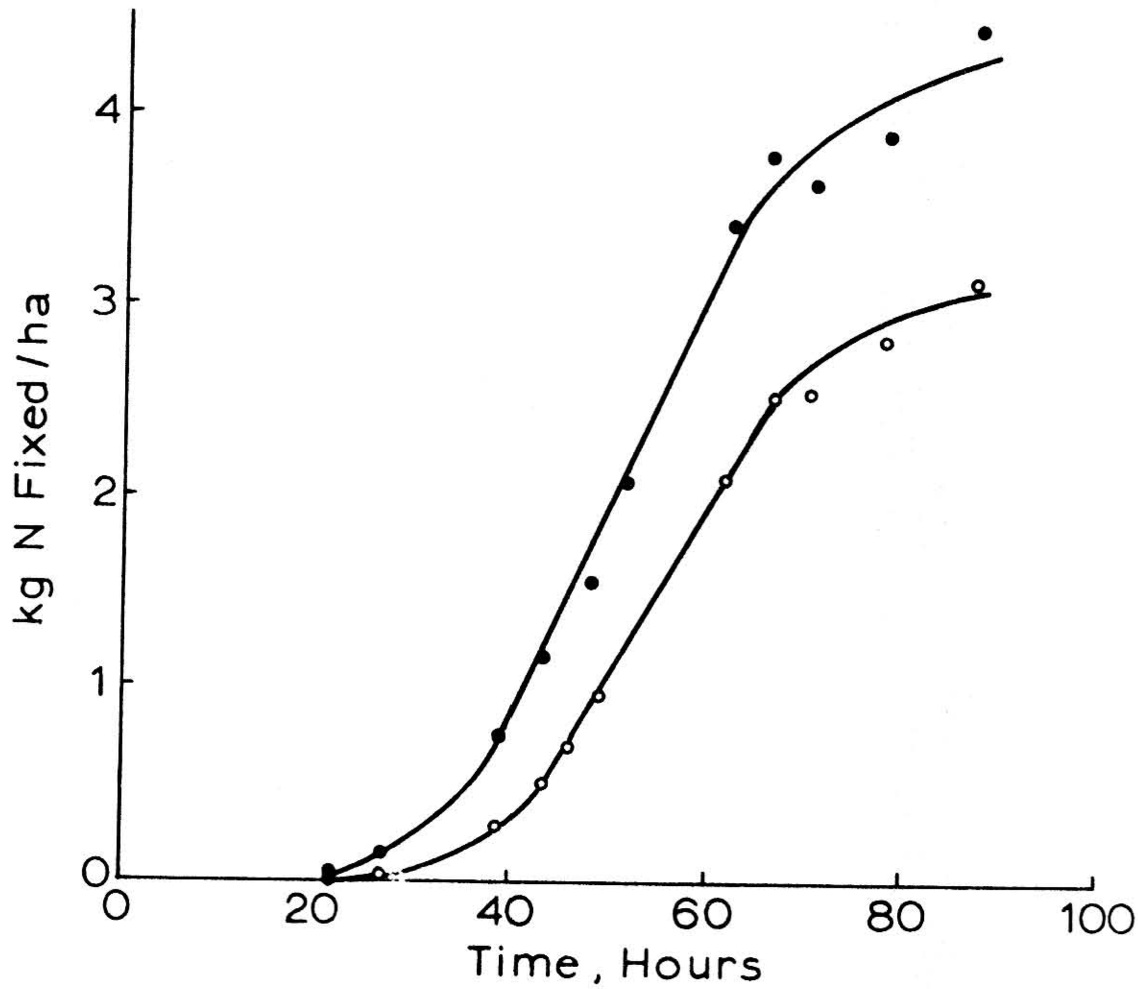
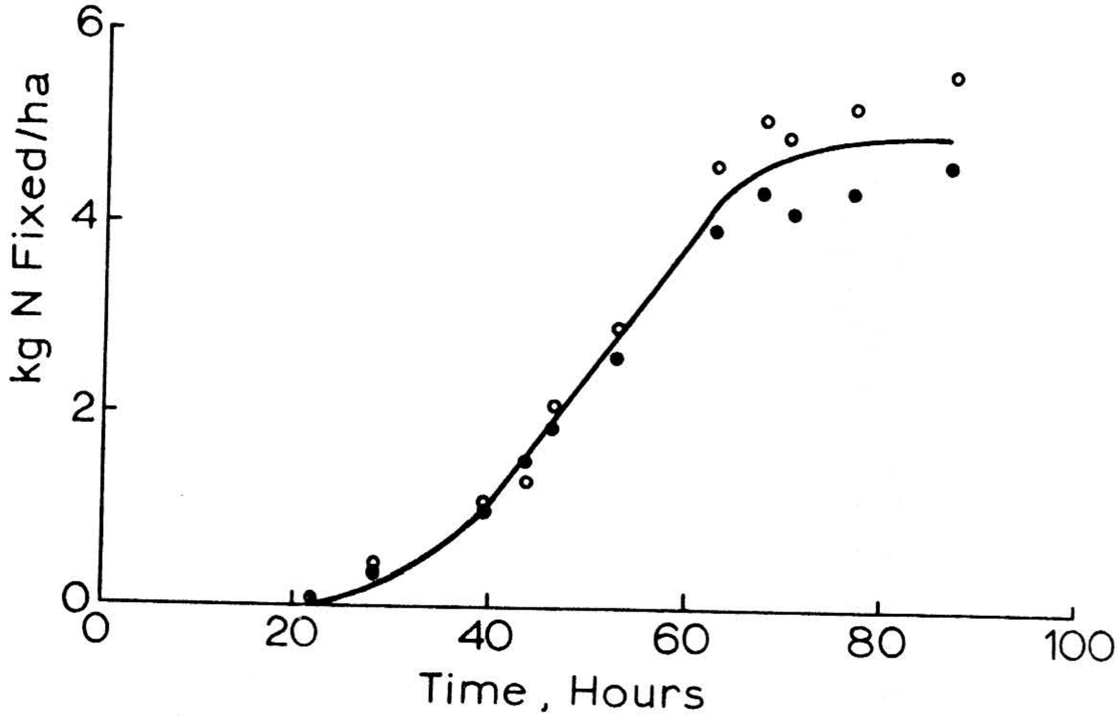


Appendix Fig. 1. Nitrogen fixation of duplicate cores of bottomland soils under saturated moisture conditions and with the addition of sucrose at the rate of 140 kg/ C/ha.

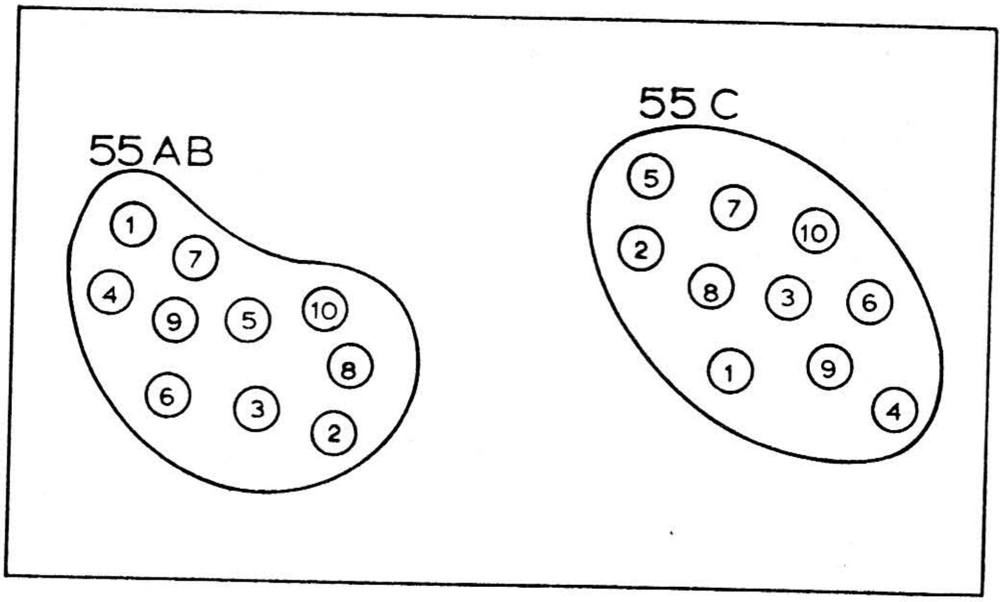


Appendix Fig. 2. Nitrogen fixation of duplicate cores of upland soil under saturated moisture conditions and with the addition of sucrose at the rate of 560 kg C/ha.

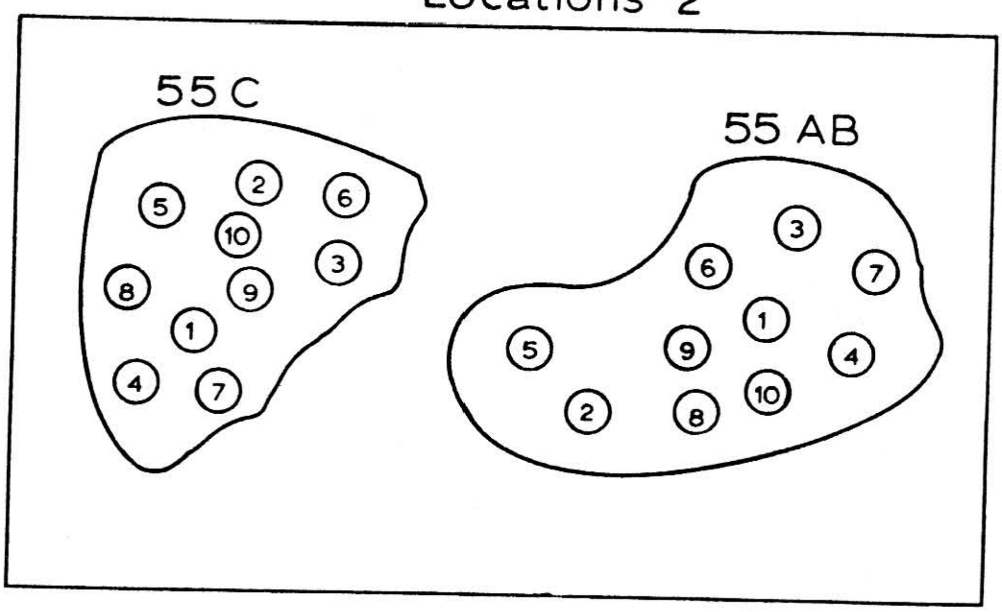


Appendix Fig. 3. Nitrogen fixation of duplicate cores of bottomland soil under saturated moisture conditions and with the addition of sucrose at the rate of 560 kg C/ha.

Locations 1



Locations 2



Appendix Fig. 4. Schematic diagram of sampling plan used in total N sampling variation study.