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Comparisons of Laboratory and Greenhouse Indexes of Nutrient Availability in Soils

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SUMMARY

Accurate indexes of the availability to plants of nutrients in soils are needed for comparisons of different chemical tests to determine which tests should be used in the laboratory for any given group of soils. This study was conducted to develop and improve techniques for obtaining such standard indexes of nutrient availability to plants. Emphasis was placed on: (a) treating the soil similarly (or uniformly) before testing in the greenhouse and laboratory and (b) avoiding treatments during the greenhouse cropping that might influence the availability of the soil nutrient being tested.

The greenhouse technique involved growing plants on undried samples of 24 different soils. Water was added directly to the soils during plant growth, but nutrients other than the one being tested were added by a method which minimized contact with the soil.

Evidence is presented to show that many factors influenced the test results obtained either in the laboratory or in the greenhouse. These included:

1. Drying of the soil samples before testing;
2. Changes in laboratory test results due to nutrient removal by the plants or to translocation of nutrients from the sand layer below the soil into the soil by the plant roots during the greenhouse cropping period;
3. Variations in the initial nitrate content of the soil samples;
4. Physical properties of the soil that interfere with root development;
5. Nutrient content of the plant seeds;
6. Varying rates of plant development and competition between plants; and
7. Plant deficiencies of nutrients other than the one in question.

When the effects of the appropriate factors were considered, the results of the greenhouse and laboratory tests for N availability in soils were highly correlated. Similarly, the greenhouse and laboratory test results for P availability were highly correlated. The data suggest that similar techniques would provide high correlations between laboratory and greenhouse tests for K availability in soils.

Comparisons of Laboratory and Greenhouse Indexes of Nutrient Availability in Soils¹

by John J. Hanway and Turgut Ozus²

The use of chemical soil tests in the laboratory to provide indexes of plant availability of nutrients in soils must be based on the premise that the chemical tests provide reliable measures of the relative availability to plants of nutrients in the different soils tested. Many different chemical tests have been developed for this purpose. Therefore, if these chemical tests are to be used in a given area, the first problem becomes one of selecting the most reliable chemical test for the soils of that area.

To determine which chemical test provides the best index of nutrient availability to plants, it is necessary to have accurate standards for comparing the different tests. These standards should be samples of the soils from the area in question and for which accurate indexes of plant availability of the nutrients have been determined. It is generally recognized that these indexes of plant availability can best be obtained in a greenhouse study where samples of the different soils can be planted to one crop and where all the samples are exposed to the same environmental conditions during the cropping period.

However, other factors have not been so generally recognized but may be of equal importance. It would appear that the following factors should be considered:

1. The soil samples be treated the same before testing in the greenhouse as they are before testing in the laboratory. Differences in pretreatment may lead to effects on the tests that are not consistent among soils. (Ideally, subsamples for testing in the laboratory should be taken at the time the soils are potted for the greenhouse experiment.)
2. The soil samples not be dried, or modified in any other way more than is essential, before testing in the greenhouse or the laboratory if they are to be representative of the field soils (2, 13, 19).³
3. Nothing be added to the soil sample before testing in the laboratory or the greenhouse or during testing in the greenhouse that might influence the availability in the soil of the nutrient being tested.

4. In the greenhouse culture, adequate amounts of nutrients, other than the one in question, be supplied to the plants so deficiencies of these nutrients do not influence absorption and utilization of the nutrient in question.

5. In the greenhouse test, the plants be grown on the soil samples long enough (but preferably with intermediate harvests) to provide a proper and adequate evaluation of nutrient availability in the soils to the plants.

This study was conducted as the initial phase in developing techniques, especially in the greenhouse, which would help evaluate the effects of these factors (21).⁴ Primary emphasis was placed on measuring N and P availability in the soils, but some observations concerning the measurement of K availability were also made.

LITERATURE REVIEW

The results of many attempts to estimate the availability of different soil nutrients to plants grown in the greenhouse and to relate these estimates of nutrient availability to the results of chemical tests in the laboratory have been reported, and such literature is too extensive to review in this bulletin. Different techniques have been used, but research studies to develop the most satisfactory methods for (a) preparing soil samples for testing in the greenhouse or laboratory and (b) conducting the greenhouse experiment have been very limited.

Nutrient availability in soil samples may be markedly changed by drying the samples. Several studies (1, 10, 11, 19, 24) have shown that drying the soil sample before analysis changed the exchangeable K level in the sample. Other studies (1, 2, 19) have shown that drying the soil sample before cropping in the greenhouse changed the availability of soil K to plants. Exchangeable K in the dried soil samples did not revert to the original undried levels on rewetting (24). Drying soil samples has been found to increase the rate of N mineralization when the samples were rewetted (4, 16, 23).⁵ Kaila (15) observed appreciable changes in the amounts

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³ Maung Khin Win. Effect of air drying on nitrate production in some Iowa soils. Unpublished M.S. thesis, Iowa State University Library, Ames, Iowa, 1959.

⁴ T. Ozus. Comparisons of laboratory and greenhouse tests for nutrient availability in soils. Unpublished M.S. thesis, Iowa State University Library, Ames, Iowa, 1964.

⁵ Maung Khin Win, op. cit.

of P soluble in a 0.03 N NH_4F + 0.025 N HCl solution as a result of drying different soils.

Nutrient availability has been shown to change in soil samples during storage. Robinson (22) observed that soluble P increased during dry storage of soil samples. Unpublished data for Iowa soils⁶ show that the amount of P extracted by the Bray (3) method increased during storage of either dried or undried soil samples and that the magnitude of this change during storage increased as the temperature of storage increased. Nitrate accumulation in soil samples during an incubation period may vary with the time of storage of the soil samples prior to incubation (14).

Many greenhouse techniques have been used for estimating the availability of soil nutrients to plants, and these estimates have often been used as the basis for comparing the results of different chemical tests in the laboratory. In most of these studies, however, the cropping period was very short, or various salts were added to the soils in the greenhouse to supply the plants with all nutrients except the one in question. The effects of these added nutrients on the availability of the one in question have rarely been considered. However, additions of CaCO_3 or of P and K fertilizers have been shown to increase the rate of N mineralization in some soils (6, 14).⁷ The availability of soil P to plants may be increased by adding lime (26) or N fertilizers (8). Additions of NH_4^+ salts may reduce the availability of soil K to plants (28).

The potential importance of considering these factors was indicated in a study conducted in the North Central Region of the United States (2). After conducting laboratory and greenhouse experiments in 1955 and 1956, an experiment was conducted in 1957 in which care was taken to (a) keep the soil samples undried before testing in the greenhouse and the laboratory, (b) obtain the laboratory sample as a subsample of the soil being potted in the greenhouse and (c) add N to the soil in the greenhouse in the NO_3^- rather than the NH_4^+ form. When this was done, plant uptake of K from the soils was highly correlated ($R^2=0.92^{**}$)⁸ with exchangeable K in the soils; whereas, in the 1955 and 1956 experiments in which these factors were not controlled, the correlation was much lower.

The 38 soil samples studied varied in texture and included samples of both surface soils and subsoils from six states in the North Central Region. The only values that deviated significantly from the general relationship were those for an organic soil, Houghton muck; all the other samples were from mineral soils.

MATERIALS AND METHODS

Soil samples of approximately 100 pounds each were collected from the 0-6 inch depth at 24 sites representing

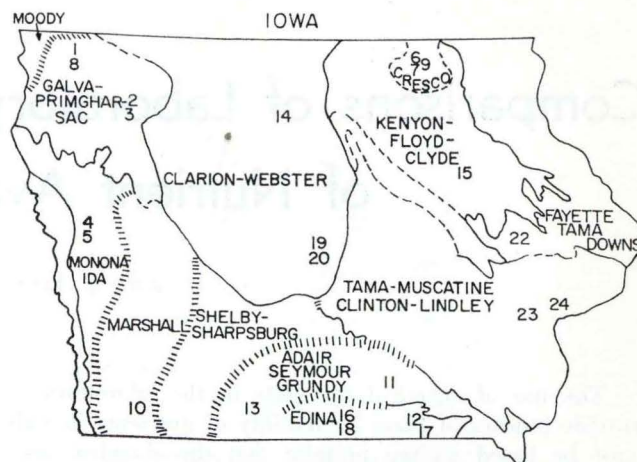


Fig. 1. Location of sites sampled with respect to soil association areas in Iowa.

17 different upland soil types in Iowa as shown in fig. 1 and described in appendix table A-1. General information on these soil types is given by Oswald, et al. (20). All samples were collected from Nov. 13 to 20, 1962. Corn (*Zea mays* L.) had been grown on all sites in 1962. All except five of the samples were from unfertilized plots on experimental farms. Each sample was screened through a 1/4-inch screen and thoroughly mixed, but was kept undried. One subsample was then screened through a 10-mesh screen and stored at 5°C. until analyzed in the laboratory where analyses of undried and air-dried portions were made. One subsample was taken for moisture determination and mechanical analyses. Other subsamples were potted and seeded on Nov. 26 and 27 for the greenhouse study.

In the greenhouse, enough clean quartz sand was placed in No. 10 cans (15.5 cm. in diameter and 17.5 cm. high) lined with polyethylene bags to make the weight of each can plus sand equal 2,000 g. An 8-inch section of plastic garden hose (1/2-inch diameter) was placed in the center of each can, extending to a depth of about 1/2 inch from the bottom of the can. Field-moist soil equivalent to 1,500 g. of oven-dry soil was then placed in each can. Common ryegrass (*Lolium multiflorum*) was seeded on the surface of the soil in all cans. Since ryegrass becomes established slowly, eight corn (*Zea mays* L.) seeds were seeded in half the cans to provide information on a plant that makes rapid initial growth but can be harvested only once. When seeding was completed, 400 g. of quartz sand was added over the seeds. The surface of this sand layer corresponded roughly to the top rim of the can. The surface layer of sand was to (a) cover the seeds, (b) provide a slow and even loss of water from the cans and prevent drying of the soil and (c) serve as basis for measuring in harvesting the ryegrass so that all plants would be cut at the same height. The arrangement of sand, soil and plants in the can is shown in fig. 2. Enough distilled water was added to each can to bring its total weight to 4,500 g. This provided water equal to a moisture content of 25 percent for the soils and 10 percent for the sand. At later dates,

⁶ Unpublished data of J. J. Hanway.

⁷ J. W. Fitts. A nitrification procedure for predicting the availability of nitrogen to corn on Iowa soils. Unpublished Ph.D. thesis, Iowa State University Library, Ames, Iowa, 1952.

⁸ ** Significant at the 1-percent level of probability.

adequate distilled water to keep the soil moist was added to the sand surface in each can. The amount of water required for each treatment was based on weights of a few of the cans with that treatment.

Nutrient additions were pipetted through the plastic tube to the sand layer below the soil. Treatments consisted of (a) none (check), (b) PK, (c) NK and (d) NPK. Beginning on April 17, 1963, an NP treatment was applied to two replicates of the previously untreated check treatment. N was added as $\text{Ca}(\text{NO}_3)_2$, P as $\text{Ca}(\text{H}_2\text{PO}_4)_2$, and K as K_2SO_4 . Stock solutions containing 3,000 ppm N, 300 ppm P and/or 2,000 ppm K were prepared. An aliquot of 20 ml. of these stock solutions pipetted into the can added 60 mg. N, 6 mg. P and/or 40 mg. K. The cans were arranged in a split-plot design with 4 replications and with treatments as the main plots and soils as the subplots.

The corn plants emerged on Dec. 2. On Dec. 8 the corn was thinned to six plants per plot by removing the two smallest plants. Four of the corn plants per can were harvested by cutting at the sand surface on Jan. 1, 30 days after emergence, when the plants had three fully emerged leaves. The remaining two corn plants per can and the ryegrass (cut at approximately 1 inch above the sand surface) in all cans were harvested on Jan. 19, 48 days after emergence. After this harvest, growth of the grass was slow where no N was added. Subsequent ryegrass harvests were made on Feb. 23 (NK and NPK), March 23 (all treatments), April 14 (NK and NPK), April 25 (check and NP), May 9 (PK) and May 25 (check and NP); 83, 111, 133, 144, 158 and 174 days after emergence, respectively. The harvested plant material was dried at 65°C, weighed and ground for chemical analysis.

After the final harvest, soil samples were taken from the center of the soil in each can. These samples were

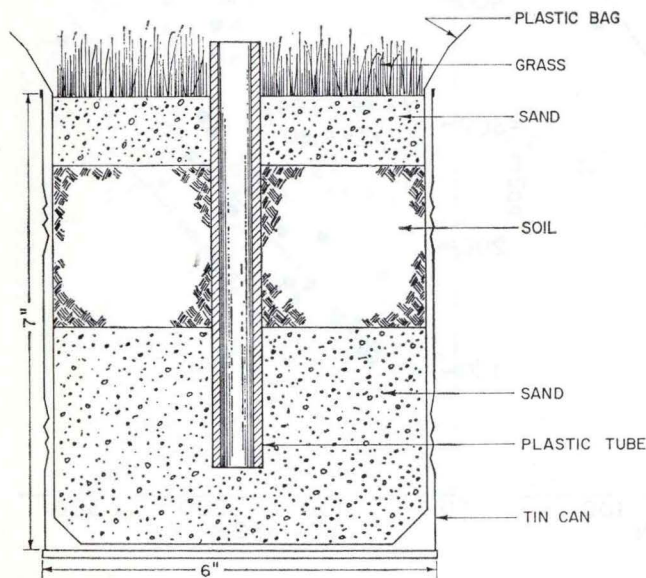


Fig. 2. Cross-section view of the can containing sand and soil as used in the greenhouse study.

screened through a 10-mesh screen, kept undried and analyzed in the laboratory.

In the laboratory, soil pH was determined with a glass electrode by using a 1:2 soil: water ratio. Mechanical analyses were made by the pipette method after destroying organic matter with H_2O_2 . Initial NH_4^+ and NO_3^- were determined by steam distillation of a 5-g. soil sample in 25 ml. of 2N KCl plus 0.15 g. of powdered, heavy MgO . The distillate was caught in boric acid and titrated to determine the NH_4^+ . Then 0.4 g. of finely ground Devarda's alloy was added to the distillation flask. After steam distilling again, the distillate was caught in boric acid and titrated to determine the NO_3^- (4). The NH_4^+ production test consisted of an anerobic incubation of 5 g. of soil in 10 ml. of H_2O at 40°C. for 1 week and then determining NH_4^+ as described (27). NO_3^- production was determined by leaching 10 g. of soil with water, incubating for 2 weeks at 35°C. and determining the NO_3^- produced (25). P was extracted with 0.025 N HCl plus 0.03 N NH_4F . One procedure ("A") for measuring P in the extract used the method of Laverty (17), extracting with a 1:10 soil:solution ratio and determining P in the extract colorimetrically using 1-amino-2-naphthol-4-sulfonic acid as a reducing agent. Another procedure ("B") for measuring P in the extract used a modification of the Bray (3) method, extracting with a 1:7 soil:solution ratio and determining P colorimetrically by using SnCl_2 as a reducing agent. K was extracted with neutral, 1 N NH_4OAc , and K in the extract was determined with a flame photometer.

Weighed samples of plant material were digested by boiling in concentrated H_2SO_4 for 24 hours. N in the diluted digest was determined by adding NaOH to an aliquot of the digest, steam distilling, catching the NH_4^+ in boric acid and titrating. P in the diluted digest was determined colorimetrically by using a vanado-molybdate procedure, and K was determined by using a flame photometer.

EXPERIMENTAL RESULTS

Soil Analyses

Analyses before cropping

The soil samples used in this study varied markedly in different properties, as shown by the laboratory analyses of the samples before the greenhouse cropping reported in table 1. Soil texture varied from a sandy loam to a clay loam, with a range between samples of from 2 to 57 percent sand and from 7 to 39 percent clay. Most of the soils were slightly acid with pH values of 6.4 to 7.0, but a few were calcareous with pH values of 8.1 to 8.3. The percentage total N, which provides an index of total organic matter, ranged from 0.077 to 0.333. Initial NO_3^- -N content at the time of potting the soils in the greenhouse varied from 5 to 49 pp2m. Rates of N mineralization varied widely as indicated by the range of from 20 to 132 pp2m NH_4^+ -N produced during a 1-week anaerobic incubation at 40°C. and by the range

Table 1. Characteristics of the soil samples at the time of potting for the greenhouse study.

Sample No.	Mechanical analyses			Soil tests ^a							
	H ₂ O	sand	clay	pH	Total N	Initial - NO ₃ -N	NH ₄ ⁺ -N production	NO ₃ ⁻ -N production	P		K
	%	>0.05 mm	<0.002 mm						A ^b	B ^b	
		%	%		%	pp2m	pp2m	pp2m	pp2m	pp2m	pp2m
1	19.5	6	22	6.7	0.197	12	62	43	20	2.7	319
2	25.2	4	25	7.1	0.294	13	74	60	50	12.0	202
3	23.8	4	28	6.6	0.262	12	66	63	25	4.4	200
4	15.6	14	7	8.3	0.080	6	58	37	5	0.5	185
5	21.8	7	18	6.9	0.167	15	84	59	47	15.0	>400
6	17.3	26	23	6.7	0.221	22	78	67	11	1.2	66
7	18.5	24	25	6.9	0.162	24	104	77	28	10.5	142
8	23.1	5	27	6.5	0.176	49	68	65	11	1.7	291
9	18.5	25	25	6.5	0.235	16	70	53	26	8.5	217
10	20.8	4	33	6.7	0.096	8	62	38	34	10.5	363
11	24.9	4	17	7.0	0.151	6	72	36	22	7.7	114
12	26.9	2	20	6.8	0.116	6	68	49	28	9.8	198
13	27.0	5	27	6.4	0.207	19	66	70	7	1.7	160
14	22.3	29	32	8.1	0.301	25	58	47	5	0.5	107
15	26.8	20	25	6.9	0.212	12	70	65	17	4.0	73
16	31.6	3	31	6.7	0.202	14	64	65	9	1.7	137
17	26.8	2	23	6.6	0.153	9	76	71	18	5.7	99
18	27.6	6	39	7.2	0.077	8	36	28	40	13.5	194
19	25.5	25	35	8.2	0.333	20	48	30	6	0.5	84
20	14.4	57	17	6.6	0.084	5	20	18	5	0.7	73
21	23.2	10	19	6.4	0.127	14	66	40	15	3.2	77
22	21.8	10	22	6.6	0.173	11	68	38	15	2.2	126
23	26.1	4	28	6.7	0.151	11	52	33	20	5.1	139
24	25.6	10	21	6.4	0.250	12	132	60	92	56.0	>400

^a NH₄⁺ and NO₃⁻ production and K were determined on undried soil samples.

^b P in extract determined by using (A) 1-amino-2-naphthol-4-sulfonic acid and (B) SnCl₂.

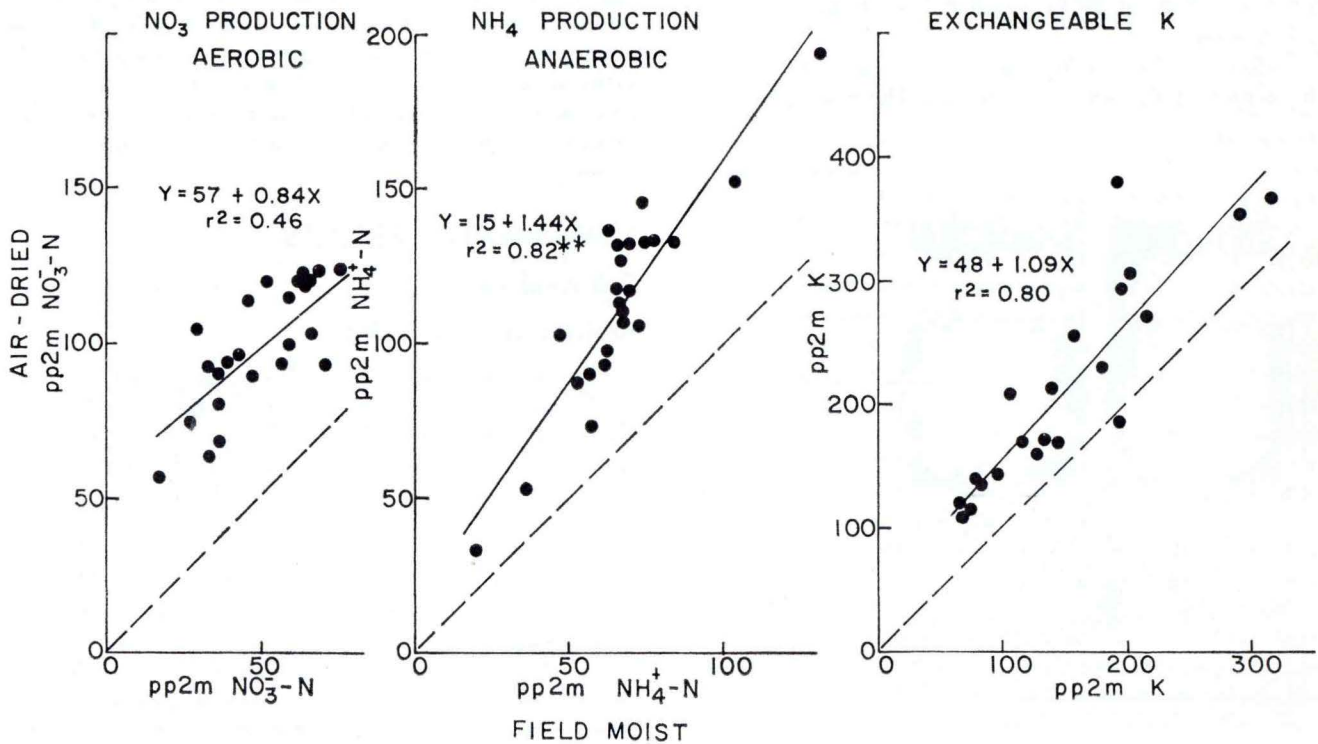


Fig. 3. Effect of air drying before analysis on the rates of aerobic and anaerobic mineralization of N and on the amount of exchangeable K in the soil samples studied.

of from 18 to 77 pp2m $\text{NO}_3\text{-N}$ produced during a 2-week incubation at 35°C . Soil tests for P varied from very low to very high levels—5 to 92 pp2m by the “A” (sulfonic acid) method and 0.5 to 56.0 pp2m by the “B” (SnCl_2) method. The absolute values obtained by the two P methods were very different but were highly correlated; omitting soil No. 24, which had a very high test by both methods, $P_A=5.7+2.71 P_B$, $r^2=0.90^{**}$. Soil tests for K varied from very low, 66 pp2m, to very high, >400 pp2m.

Effect of air-drying the soil samples

Although the basic greenhouse and laboratory studies were conducted with undried soil samples, analyses of air-dried samples were made to determine the effect of drying on these particular soil samples. Air drying the samples before laboratory analysis increased soil pH by 0.1 to 0.2 pH units; $\text{pH (air dried)} = -0.41 + 1.08 \text{ pH (undried)}$, $r^2=0.89^{**}$. Air drying had no appreciable effect on the values obtained from the laboratory test for P for the “B” method; $P(\text{air dried}) = 0.0 + 1.0(\text{undried})$, $r^2=0.89^{**}$. As shown in fig. 3, air drying the soils before testing in the laboratory increased the aerobic and anaerobic rates of mineralization of N during the incubation period and, usually, increased the amounts of exchangeable K in these soil samples, but the degree of change in the N and K test results because of drying was not the same for all soils.

Effect of cropping on laboratory test results

Samples of the soils were collected from some of the greenhouse treatments at the end of the cropping period. The pH of the soils was not changed by greenhouse cropping. Soil pH was not influenced by the nutrient additions to the sand layer below the soil; pH after cropping was the same in samples from the check treatment and the NPK treatment.

Nitrate production during a 2-week incubation of undried soil samples was not changed appreciably by the cropping, nor did the nutrient additions to the sand layer below the soil have any influence on the $\text{NO}_3\text{-N}$ production during incubation after cropping. The average $\text{NO}_3\text{-N}$ production after cropping was 52, 56 and 55 pp2m for the check, PK and NPK treatments, respectively, as compared with an average of 51 pp2m before cropping. However, there was a tendency for $\text{NO}_3\text{-N}$ production to increase in soils with low initial levels and to decrease in soils with higher initial levels as indicated by the regression equation: $N(\text{after}) = 27 + 0.55N(\text{before})$, $r^2=0.58^{**}$.

The P soil-test values were reduced by cropping where no P was added to the sand layer below the soil, except at very low levels of P where there was little or no change from cropping (fig. 4-A). The decrease in P soil-test levels was greater when NK was added to the sand below the soil than when nothing was added. When NPK was added to the sand layer below the soil, the P soil test

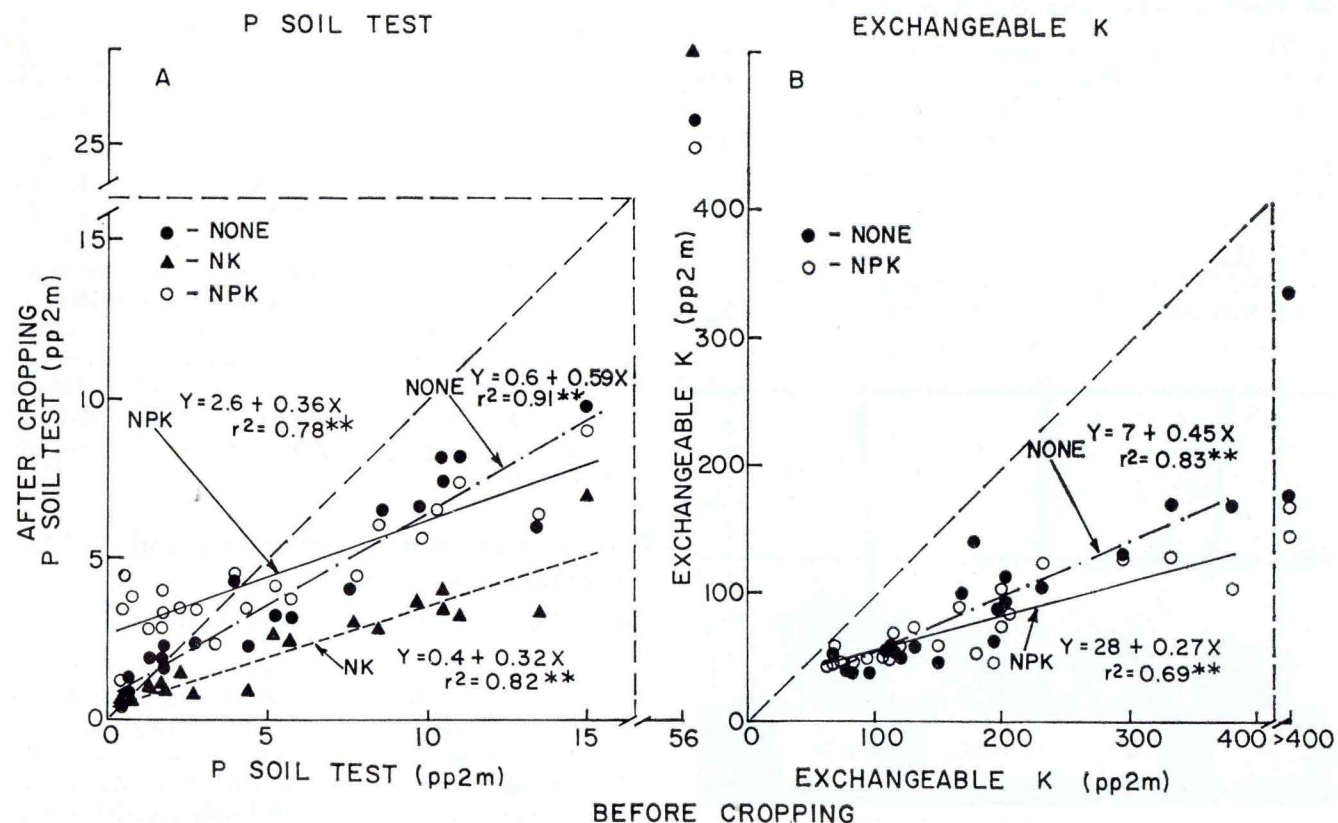


Fig. 4. The influence of nutrient additions to the sand layer below the soil in greenhouse pots on P soil-test values (A) and exchangeable K values (B) in the soil samples after cropping in relation to the values before cropping.

increased at low levels of P in the soil and decreased at higher levels of soil P. It appears that some of the P taken up by the plant roots in the sand layer below the soil was lost to the soils with low levels of "available" P, but at higher levels of P in the soil, the plants absorbed soil P even though P was supplied in the sand below the soil. The slopes of the regression lines relating P soil tests before and after cropping are very similar for the NK and NPK treatments ($b=0.32$ and 0.36 , respectively), but the intercepts at $X=0$ are different ($Y=0.4$ and 2.6 , respectively). This indicates that an equilibrium may become established between the level of P in the soil and the level of P added to the sand below the soil.

Exchangeable K was reduced in all soils by cropping as is shown in fig. 4-B. The decrease was proportional to the amount of exchangeable K in the soils before cropping and was greater for the NPK treatment in the greenhouse than for the check to which no nutrients were added to the sand layer below the soil. (The NPK treatment markedly increased plant growth.) However, exchangeable K was not reduced below 40 to 50 pp2m in any of the soils. It appears that exchangeable K may be reduced to near this minimum level in all these soils by continued cropping. (Part of the K measured here as exchangeable K after cropping was K present in the plant roots remaining in the soil and was not strictly exchangeable soil K.)

General Effects of Nutrient Additions on Plant Growth and Nutrient Uptake

The effect of the nutrient additions on the growth of the ryegrass is illustrated in fig. 5 for a Clarion soil that was very deficient in N and P. Dry matter yields and percentages of N, P and K in the plants of the different harvests averaged over all soils are reported in table 2.

The N, P and K added to the sand layer below the soil in the cans in the greenhouse had comparatively little effect on the dry weight or P content of the corn plants harvested on Jan. 1, 30 days after emergence, but did increase the N and K contents of the corn plants

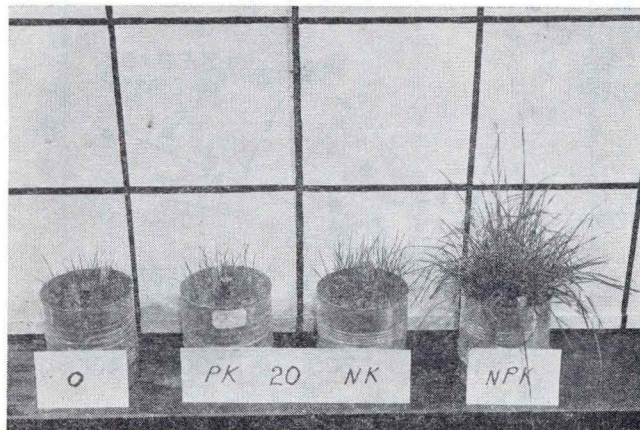


Fig. 5. Growth of ryegrass on a Clarion soil, soil No. 20 which was deficient in N and P, as influenced by nutrient additions to the sand layer below the soil, Feb. 18, 1963.

Table 2. Average dry matter yields and percentages of N, P and K in the corn and ryegrass plants at different harvests from the different treatments in the greenhouse (average of all soils).

Harvest *		Treatment	Dry weight g./pot	%N	%P	%K
Date	Crop					
1/1	Corn	None	0.50	2.24	0.33	2.91
		PK	0.48	2.18	0.36	3.42
		NK	0.59	3.18	0.26	3.72
		NPK	0.64	3.25	0.23	3.76
1/19	Corn	None	0.42	2.71	0.32	4.07
		PK	0.40	2.69	0.44	4.36
		NK	0.61	5.22	0.19	4.43
		NPK	0.75	5.70	0.27	4.82
1/19	Grass (with corn)	None	0.46	1.43	0.18	2.19
		PK	0.62	1.43	0.28	2.92
		NK	1.18	2.59	0.12	2.86
		NPK	1.40	2.58	0.12	2.48
1/19	Grass	None	0.74	2.67	0.32	3.37
		PK	0.82	2.49	0.40	3.41
		NK	1.64	4.90	0.19	3.99
		NPK	2.06	5.30	0.25	4.08
2/23	Grass	NK	2.28	4.10	0.15	3.78
		NPK	3.18	3.55	0.19	3.22
3/23	Grass	None	0.43	2.04	0.33	3.23
		PK	0.50	1.91	0.46	3.57
		NK	1.68	3.83	0.14	3.89
		NPK	2.14	2.80	0.19	3.37
4/14	Grass	NK	2.85	2.90	0.14	3.08
		NPK	3.54	2.13	0.15	2.67
4/25	Grass	None	0.32	1.56	0.36	1.95
		NP	1.09	2.94	0.26	2.14
5/9	Grass	PK	1.22	1.38	0.41	1.93
5/25	Grass	None	1.06	1.47	0.34	1.94
		NP	2.77	2.36	0.21	2.16

as shown in fig. 6. However, 18 days later the dry weights of the corn and grass and the uptake of N, P and K were markedly increased by the nutrient additions below the soil layer. The continued effect of the added nutrients, especially N and P, on dry matter and nutrient accumulation in the grass at succeeding harvests is evident in fig. 7. Periodic additions of N and P exceeded average removal, so there should have been adequate amounts of these nutrients available in the sand layer below the soil throughout the study. However, where N was added, the average removal of K by the grass did exceed the amounts of K added.

Relationships Between Laboratory and Greenhouse Test Results

Results from the first greenhouse harvests

Nitrogen. The $\text{NO}_3\text{-N}$ in the soils at the time of potting in the greenhouse had a controlling influence on growth and N uptake by the plants during the early period of cropping when the plants were becoming established. As shown in fig. 8A, the total N uptake by the corn and grass up to Jan. 19 was highly correlated with the initial NO_3 content of the soil. Where grass only was grown, the correlation between N uptake for these

Fig. 6. Dry weight and N, P and K uptake in the above-ground parts of the corn and ryegrass harvested on Jan. 1 and Jan. 19 as influenced by the nutrient additions (average of all soils).

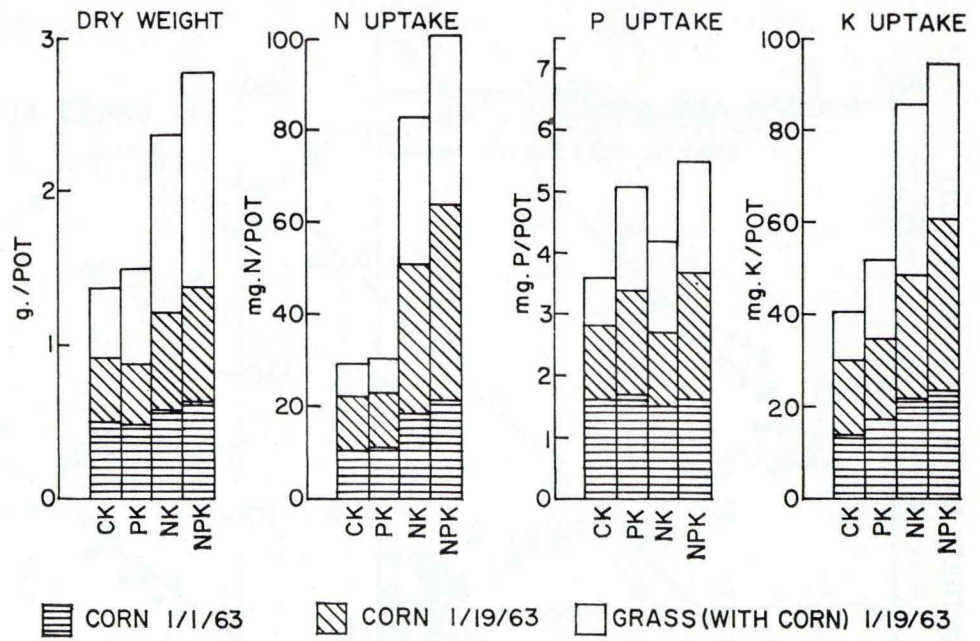
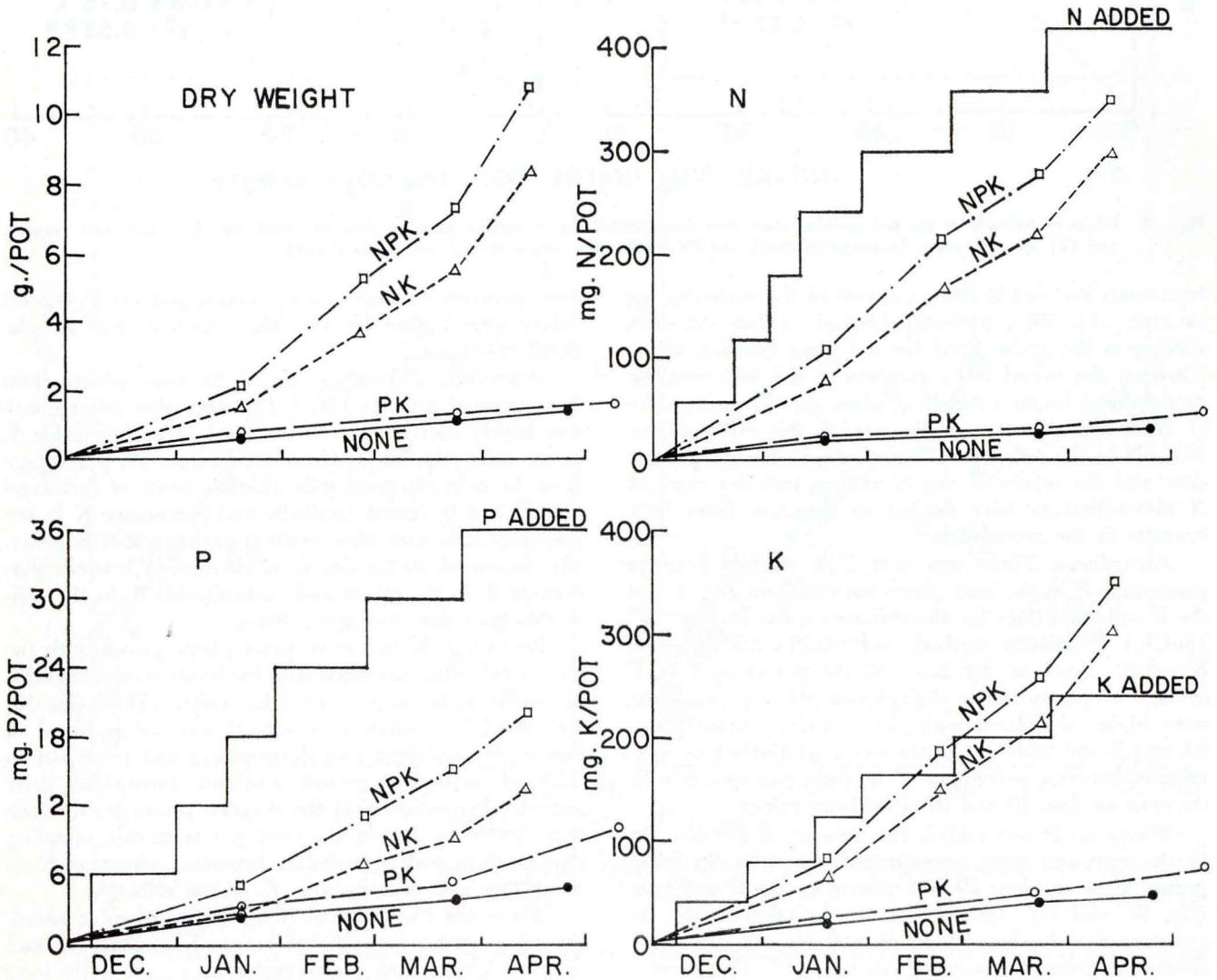


Fig. 7. Amounts of N, P and K added to the sand layer below the soil in relation to dry weight and N, P and K accumulation in the above-ground parts of the ryegrass plants in the greenhouse experiment (average of all soils).



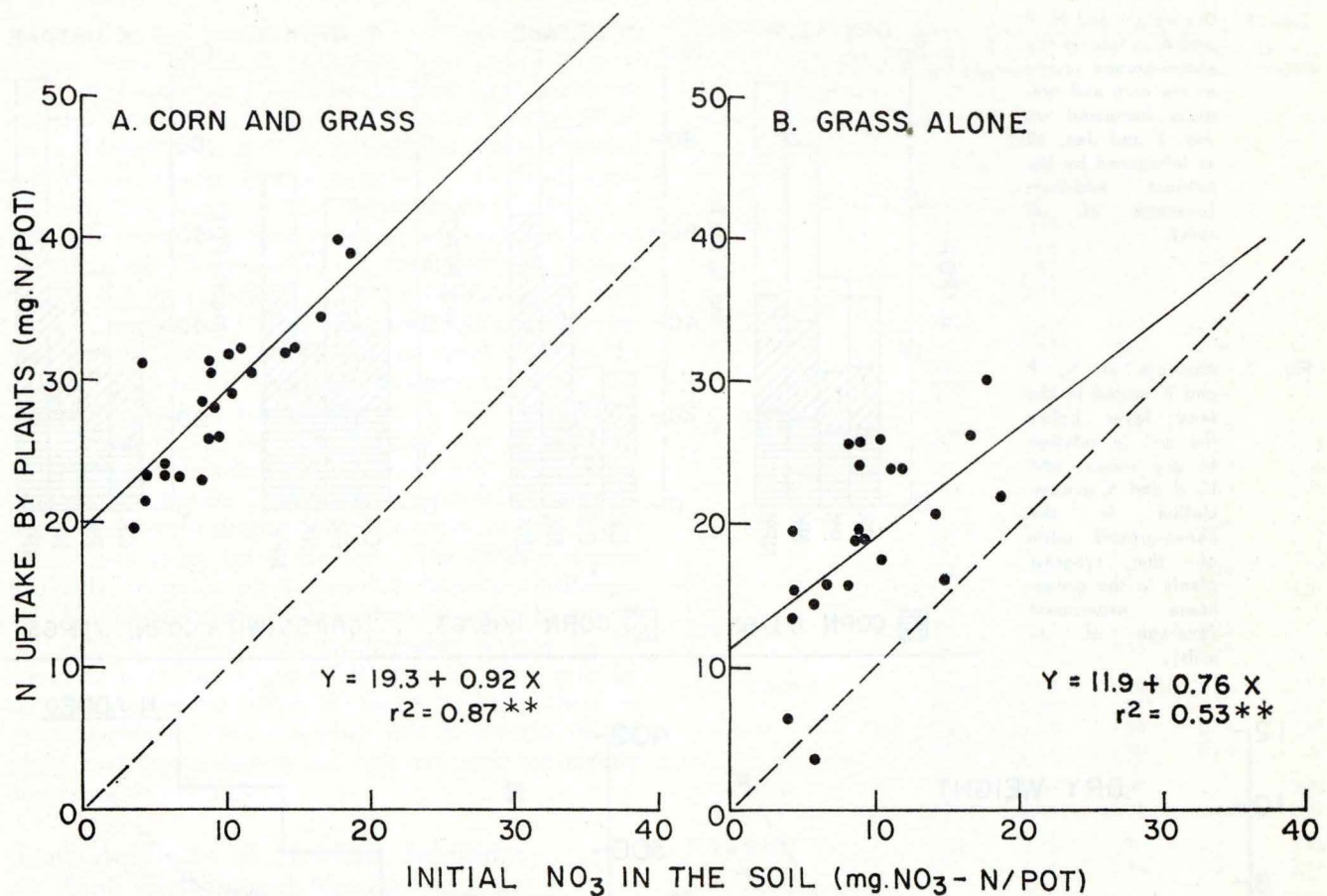


Fig. 8. Effect of NO₃-N in the soil samples when they were potted on N uptake prior to Jan. 19, 1963, by: (A) corn and ryegrass and (B) ryegrass alone (average of check and PK treatments, values for soil No. 8 not shown).

treatments and initial NO₃ contents of the soils was not as high (fig. 8B), probably because of less extensive rooting of the grass. Even for the grass growing alone, however, the initial NO₃ contents of the soils was the predominant factor controlling plant growth and uptake of N during this period. Because of this effect of the NO₃-N in the soils, correlations between the greenhouse data and the results of the laboratory tests for rates of N mineralization were limited to the data from later harvests in the greenhouse.

Phosphorus. There was very little relation between percentage P in the corn plants harvested on Jan. 1 and the P soil-test values for the different soils; for the "B" (SnCl₂) P soil-test method, %P = 0.29 + 0.0068P soil, R² = 0.22. However, by Jan. 19, the percentages of P in the corn plants and in the ryegrass plants grown alone were highly correlated with the P soil-test values (fig. 9A and B and table 3). There was a relatively poor correlation between percentage P in the grass grown with the corn on Jan. 19 and the P soil-test values.

Where no P was added, the amount of P taken up by the corn and grass grown together and by the grass grown alone on Jan. 19 was related to the P soil tests (fig. 9C and D). (Data were not included in the regressions for the heavy-textured soil No. 18 on which growth of grass was poor at this harvest.) The correla-

tions between P uptake by the plants and the P soil-test values were higher for the NK treatment than for the check treatment.

Potassium. Percentage K in the corn plants from the untreated pots on Jan. 1 (30 days after emergence) was highly correlated with the level of exchangeable K in the soils (fig. 10A). However, by Jan. 19, percentage K in the corn plants on soils with low levels of exchangeable K had increased markedly and percentage K in the plants on soils with high levels of exchangeable K generally decreased, so the degree of correlation between percentage K in the plants and exchangeable K in the soils at this later date was much lower.

Percentage K in the ryegrass plants grown with the corn was highly correlated with the levels of exchangeable K in the soils on Jan. 19 (fig. 10B). (The value for soil No. 18, a heavy, clay subsoil was not included in this regression since root development was restricted in this soil, and plant growth was slow during this early period.) Percentage K in the ryegrass plants grown alone was similar to that in the corn plants at this sampling date, with a poor correlation between percentage K in the plants and exchangeable K in the soils.

Where the PK, NK and NPK treatments were added, the relationships between percentage K in the corn and ryegrass plants and exchangeable K in the soils were

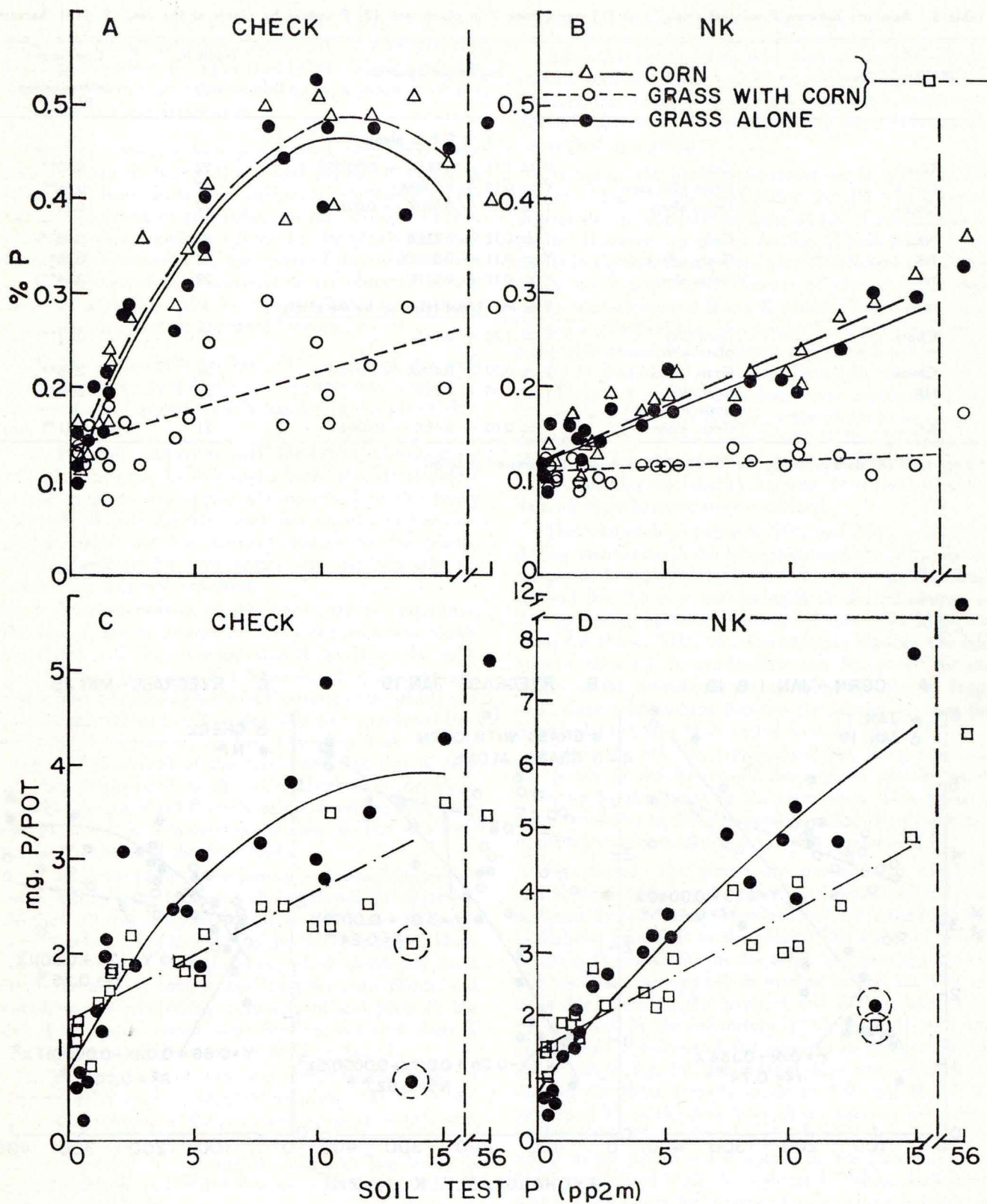


Fig. 9. Relation between P soil-test values (A method) and the percentage P and P uptake by corn and ryegrass plants harvested on Jan. 19.

A and C = check treatment.

B and D = NK treatment.

(Data for soil No. 24, with a soil test of 56 pp2m, and the circled values, representing soil No. 18, a very heavy-textured sub-soil, were not included in the regressions.)

Table 3. Relations between P soil-test values* and (1) percentage P in plants and (2) P uptake by plants at the Jan. 19, 1963, harvest.

Treatment	Crop	Regression equation	Number of soils	Coefficient of determination R ² or r ²
Y = %P in plants				
Check.....	Corn	$Y = 0.11 + 0.066X - 0.0029X^2$	22	0.90**
Check.....	Grass with corn	$Y = 0.18 + 0.0080X$	22	0.46**
Check.....	Grass alone	$Y = 0.09 + 0.970X - 0.0033X^2$	22	0.92**
NK.....	Corn	$Y = 0.12 + 0.012X$	22	0.88**
NK.....	Grass with corn	$Y = 0.11 + 0.0012X$	22	0.20*
NK.....	Grass alone	$Y = 0.12 + 0.011X$	22	0.84**
Y = mg. P/pot taken up by the plants				
Check.....	Corn plus grass with corn	$Y = 1.28 + 0.14X$	21	0.77**
Check.....	Grass alone	$Y = 0.77 + 0.454X - 0.016X^2$	21	0.75**
NK.....	Corn plus grass with corn	$Y = 1.49 + 0.21X$	21	0.84**
NK.....	Grass alone	$Y = 0.80 + 0.49X - 0.0063X^2$	21	0.91**

* P soil tests (X) determined by the "B" (SnCl₂) method and expressed as pp2m.

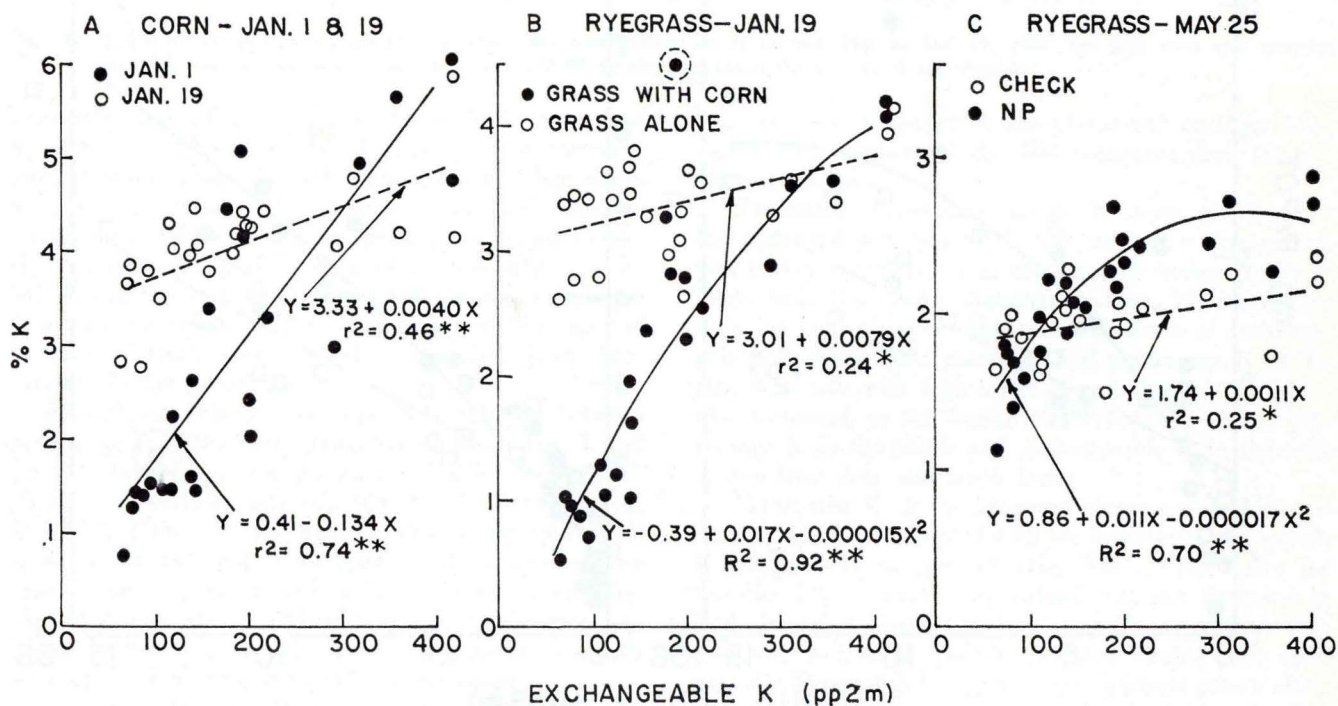


Fig. 10. Relation between exchangeable K in the undried soils and the percentage K in the plants.

- A. Corn, check treatment, Jan. 1 and Jan. 19.
 - B. Ryegrass alone and with corn, check treatment, Jan. 19.
 - C. Ryegrass, check and NP treatments, May 25.
- (Circled value in 10B for soil No. 18 was not included in the regression.)

similar to those from the untreated checks, but with somewhat more variability. Variability in root penetration into the added K in the sand below the soil probably accounts for this greater variability in K content of the plants from these treatments.

Resumé—Various factors appeared to influence the results obtained during this initial period of cropping when the plants' roots were becoming established in the soil and in the sand layer below the soil. Because of this, plant growth or nutrient uptake by the plants may not be highly correlated with the levels of any one nutrient in the soils, or, if good correlations are observed at any one time, they may exist for only a relatively short time. The following factors appeared important:

1. The differences in $\text{NO}_3\text{-N}$ contents of the soils when they were potted was a very important factor influencing plant growth and N uptake by the plants during this period.

2. Physical properties may limit root development and plant growth. In this study, early root development of the ryegrass was apparently restricted in the heavy clay subsoil (soil No. 18), and this resulted in low dry-matter yields and low nutrient uptake by the plants. Development of the corn plants was not seriously restricted by this soil condition.

3. Nutrient content of the seeds may be important. On Jan. 1, the K content of the corn plants was highly correlated with the exchangeable K levels in the soils. But, 19 days later, this K relationship had disappeared, and a new relationship between P content of the plants and the soil-test values for P in the soils had appeared. Possibly the K relationship on Jan. 1 resulted from the relatively low K content of the corn seeds, but this K relationship disappeared as the plants became N deficient. Possibly the plant-soil P relationship developed later than the plant-soil K relationship because of the relatively higher P content of the corn seeds.

4. Rate of plant development and competition between plants may influence the relationships observed. On Jan. 19, the ryegrass grown alone showed the same type of relationship to soil P as did the corn plants. However, grass grown with the corn, which developed more slowly because of competition from the corn plants, did not show this relationship between soil and plant P, but did show a good relationship between soil and plant K similar to that observed in the corn on Jan. 1.

5. A deficiency of any one nutrient may result in unusual relationships for other nutrients. As an N deficiency developed in the corn and grass grown on soils where no nutrients were added, percentage K in the plants appeared to increase on soils with low levels of exchangeable K and to decrease on soils with high levels of exchangeable K.

Because of the variability from these factors, the results obtained from this initial period of crop growth in the greenhouse are of limited value as standard estimates of the relative availability of the soil nutrients to plants.

Results from later greenhouse harvests

After the grass plants had become established and various inconsistencies had been removed in the initial harvests, the results of different subsequent harvests were similar. Therefore, the results of all later harvests are considered as a group.

Nitrogen. The greenhouse treatment in which P and K were added to the sand below the soil was designed to provide the standard estimate of soil N availability to plants. However, as shown in fig. 7 and table 2, the plants in the greenhouse were very N deficient on all soils where no N was added. Because of this severe N deficiency, the addition of P and K without N had little effect on plant growth. Thus, the results from the check treatment with no nutrient additions also served as a measure of soil N availability to plants. Therefore, the results of the check and the PK treatments were averaged to provide the measure of N uptake by the plants to be used for developing the desired relationships. Results from the March 23 (check and PK), April 25 (check), May 9 (PK) and May 25 (check) harvests were similar, so the data for these harvests were combined.

The relationships between NO_3 and NH_4 production during incubation in the laboratory and the N uptake by the plants in the greenhouse are shown in fig. 11. (Data for soil No. 24 were not included in the calculation of the regression equations because of this soil's high test levels for P and $\text{NH}_4\text{-N}$.) Correlations between the laboratory tests for N availability and N uptake by the plants in the greenhouse were highly significant. However, these relationships between the laboratory tests for N and plant uptake of N varied with the levels of "available" P in the soil. The higher the P test, the greater was the N test in the laboratory for a given amount of N taken up by the plants in the greenhouse. This effect is more consistent in the relationship with NH_4 production in the laboratory than it is with NO_3 production.

Phosphorus. The greenhouse treatment in which N and K were added to the sand layer below the soil was designed to provide the standard test for estimating the availability of soil P to the plants. The effect of differences in P availability in the soils (as measured by the soil test) on plant growth is evident in fig. 12. The relationships between the levels of soil P as measured by the soil test in the laboratory ("A" method) and the growth and P content of the ryegrass in the greenhouse are shown in fig. 13. The relationships were similar for each of the three harvests made on Feb. 23, March 23 and April 14, so the data from all the harvests were combined in this figure. Dry weights of the plants, percentage P in the plants and P uptake by the plants were all highly correlated with the soil-test P values, indicating that the laboratory test provided an excellent estimate of plant availability of P in these soils. All the relationships are curvilinear, but the relationship for the dry weight of the plants appears to reach a maximum value, whereas those for percentage P and P uptake continue to increase over the range of P values obtained in these soils.

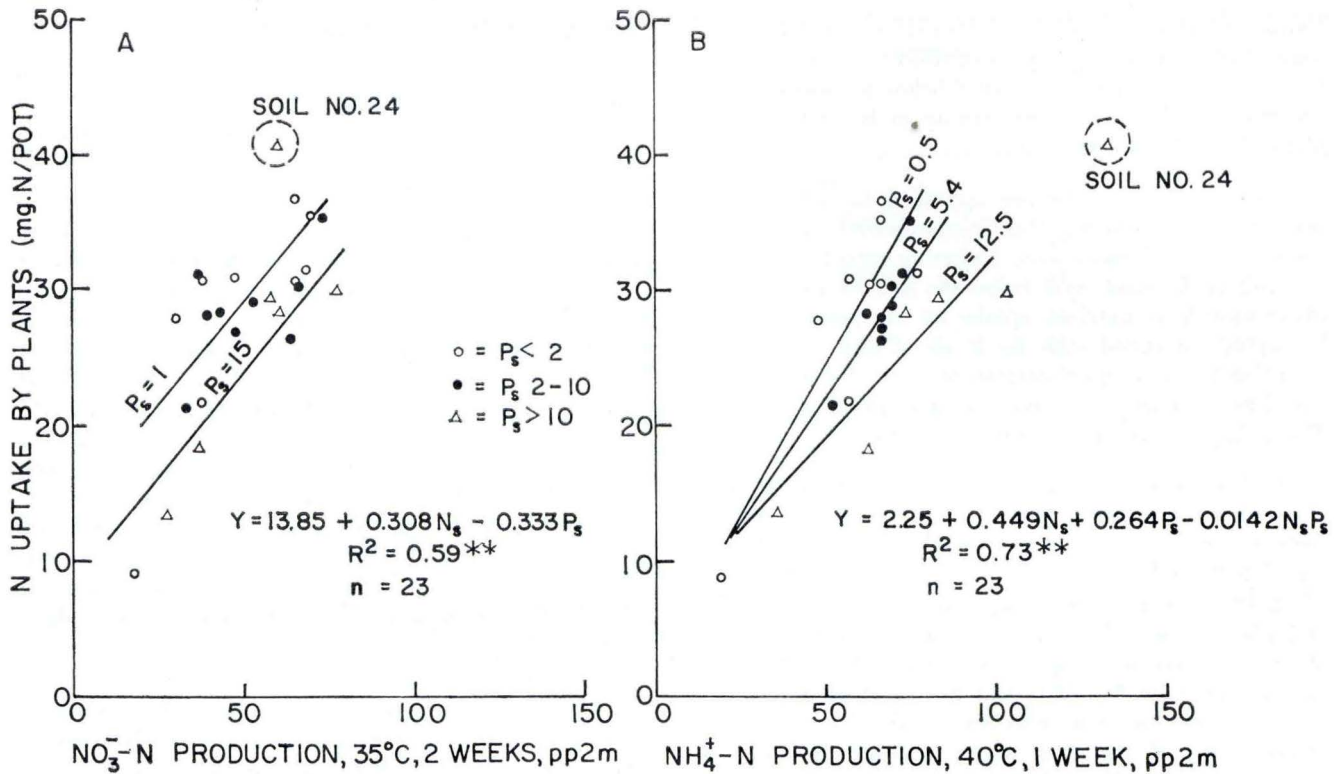


Fig. 11. Relation between N uptake by ryegrass in the greenhouse and the rate of release of NO_3^- and NH_4^+ nitrogen during incubation in the laboratory (average of check and PK treatments; totals of Mar. 23, Apr. 25, and May 9 and May 25 harvests).

A. Aerobic NO_3^- production.

B. Anaerobic NH_4^+ production.

(Soil No. 24 was not included in the regressions. $Y =$ mg. N/pot, $N_s = NO_3^-$ -N or NH_4^+ -N, $P_s =$ soil test P, pp2m, B method.)

The relationships between the P soil-test results by the "B" ($SnCl_2$) method and the dry weights and P contents of the grass for the combined data of the different harvests from the check, PK, NK and NPK treatments are shown in fig. 14.⁹ The regression equations and coefficients of determination for all these relationships are reported in table 4.

⁹ The relationships for the "B" method and the NK treatment are reported in detail elsewhere (21).

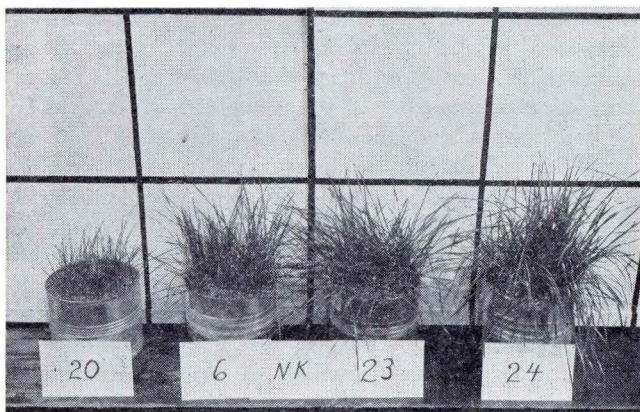


Fig. 12. Growth of ryegrass in the greenhouse as influenced by P availability in the soils, Feb. 18, 1963. N and K were added to all pots. P soil tests ("B" method) were 0.7, 1.2, 5.1 and 56.0 pp2m for soils 20, 6, 23 and 24, respectively.

Where no N was added to the sand below the soil (check and PK treatments), plant growth was poor and was not related to the P soil-test values. However, the percentage P in the plants from both of these treatments was highly correlated with the P soil-test values. Percentage P in the plants was higher for the PK treatment than where no nutrients were added, except at high soil-test levels, and was much higher than where NK or NPK was added to the pots, except at very low soil-test P values. Total plant uptake of P from the untreated and PK-treated pots was related to the soil-test P values, but the correlation coefficients were not high.

Where NPK was added to the sand layer below the soil, plant yields were markedly increased on soils with low levels of available P. However, yields were still correlated with the P soil-test values ($r^2 = 0.52^{**}$). The NPK treatment resulted in much higher yields than did the NK treatment at low P soil-test values, but at higher P soil-test values, the yields were similar. Possibly additions of larger amounts of P in the NPK treatment would have resulted in similar yields on all soils irrespective of the P level in the soils. The NPK treatment increased percentage P in the plants to only slightly higher values than were found for the NK treatment. The percentage P in the plants and mg. P taken up by the plants from the NK and the NPK treatments were highly correlated with the P soil-test values ($r^2 = 0.90^{**}$ and 0.96^{**} for NK and 0.91^{**} and 0.94^{**} for NPK, respectively). The re-

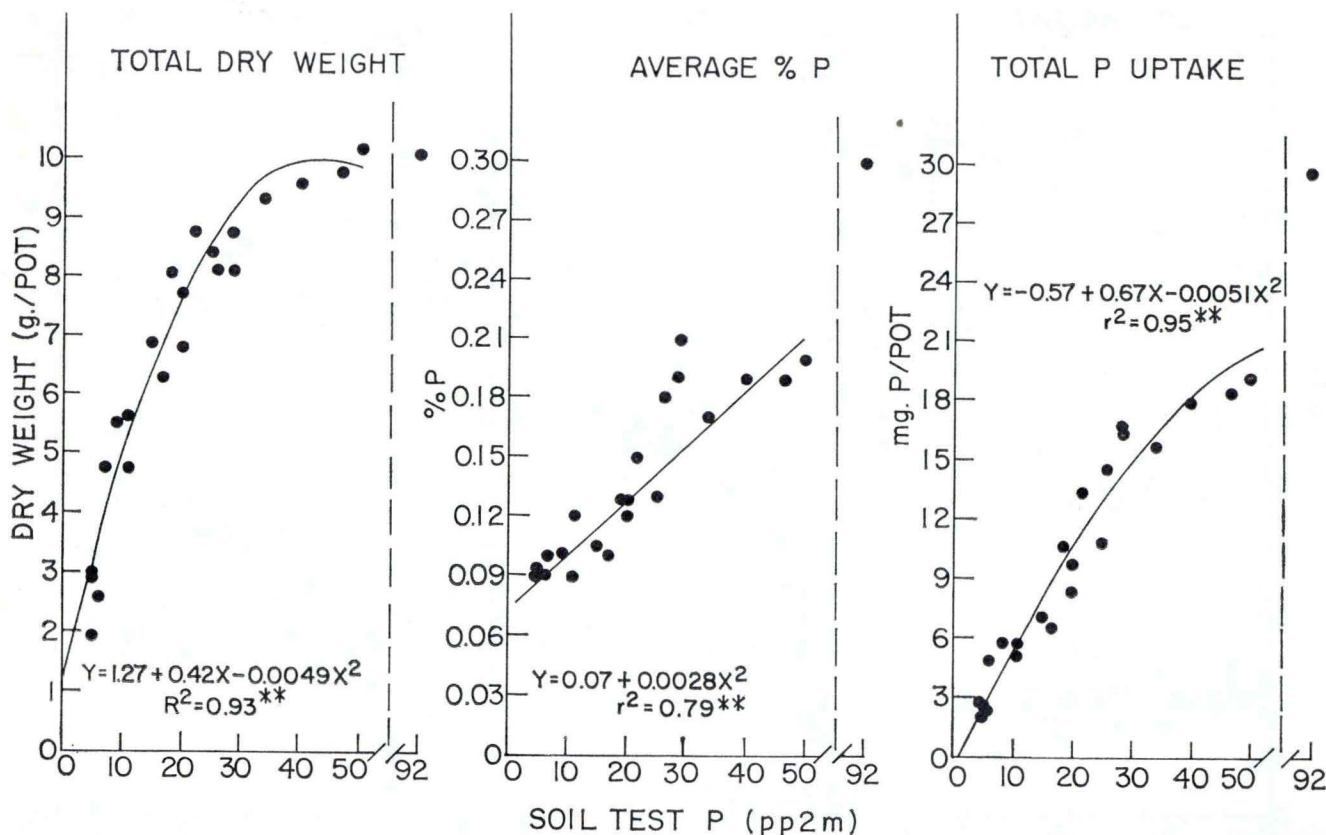


Fig. 13. Dry weights, percentage P and mg. P uptake in the above-ground parts of ryegrass plants in relation to laboratory soil tests for P in the different soils. (P determined in the laboratory by use of 1-amino - 2-naphthol - 4-sulfonic acid.) N and K added to all pots. Combined data for Feb. 23, March 23 and April 14 harvests. (Data for soil No. 24, with a very high P test, was not included in the regression equation.)

gression lines relating P uptake to P soil-test values for the NPK and NK treatments were very nearly parallel.

Potassium. After the initial period of growth in the greenhouse, the plants grown in pots with the PK, NK and NPK treatments all obtained adequate K from the nutrients added to the sand layer below the soils. The K content of these plants varied between 2 and 4 percent.¹⁰ Where nothing was added to the sand layer below the soils, the plants became very N deficient, and, as with the corn and the grass grown alone and harvested on Jan. 19, there was no relationship between the K content of the plants and exchangeable K in the soils.

Later, on April 17, the NP treatment was begun using two replicates of the check treatment to determine whether a relationship would again develop between the K contents of the plants and exchangeable K in the soils when the N and P deficiencies were corrected. As shown in fig. 10C, a good relationship between percentage K in the plants and exchangeable K in the soils did develop where N and P were added, whereas the correlation between these variables was still very low in the two replicates where the check treatment was continued. The NP treatment increased plant growth on all soils, reduced percentage K in the plants on soils with low levels of exchangeable K, and increased percentage K in the plants on soils with high levels of exchangeable K.

¹⁰ T. Ozus, op. cit.

Table 4. Relation between yield of dry matter, percentage P in plants and yield of P (Y) and the P soil-test values of the soils (X) as influenced by nutrient additions to the sand layer below the soil.*

Treatment	Regression equation	Coefficient of determination (r ² or R ²)
Dry weight of plants (g./pot)		
None.....	Y = 1.85 - 0.146X	0.01
PK.....	Y = 1.85 - 0.0286X	0.12
NK.....	Y = -0.69 + 5.45 X ^{1/2} - 0.74X	0.93**
NPK.....	Y = 8.06 + 0.35X	0.52**
%P in the plants		
None.....	Y = 0.129 + 0.051X - 0.0016X ²	0.88**
PK.....	Y = 0.292 + 0.041X - 0.0016X ²	0.82**
NK.....	Y = 0.076 + 0.013X - 0.00031X ²	0.90**
NPK.....	Y = 0.104 + 0.018X - 0.00067X ²	0.91**
Yield of P (mg./pot)		
None.....	Y = 1.96 + 1.25X - 0.064X ²	0.69**
PK.....	Y = 4.96 + 0.75X - 0.041X ²	0.41**
NK.....	Y = 1.86 + 1.95X - 0.054X ²	0.96**
NPK.....	Y = 8.16 + 1.69X - 0.054X ²	0.94**

* Combined data for Feb. 23, March 23, April 14 and 25, and May 9 harvests. P soil tests by the "B" (SnCl₂) method. Data for soils No. 18, a heavy textured subsoil, and No. 24 with a P soil test of 56 pp2m were not included in these regressions.

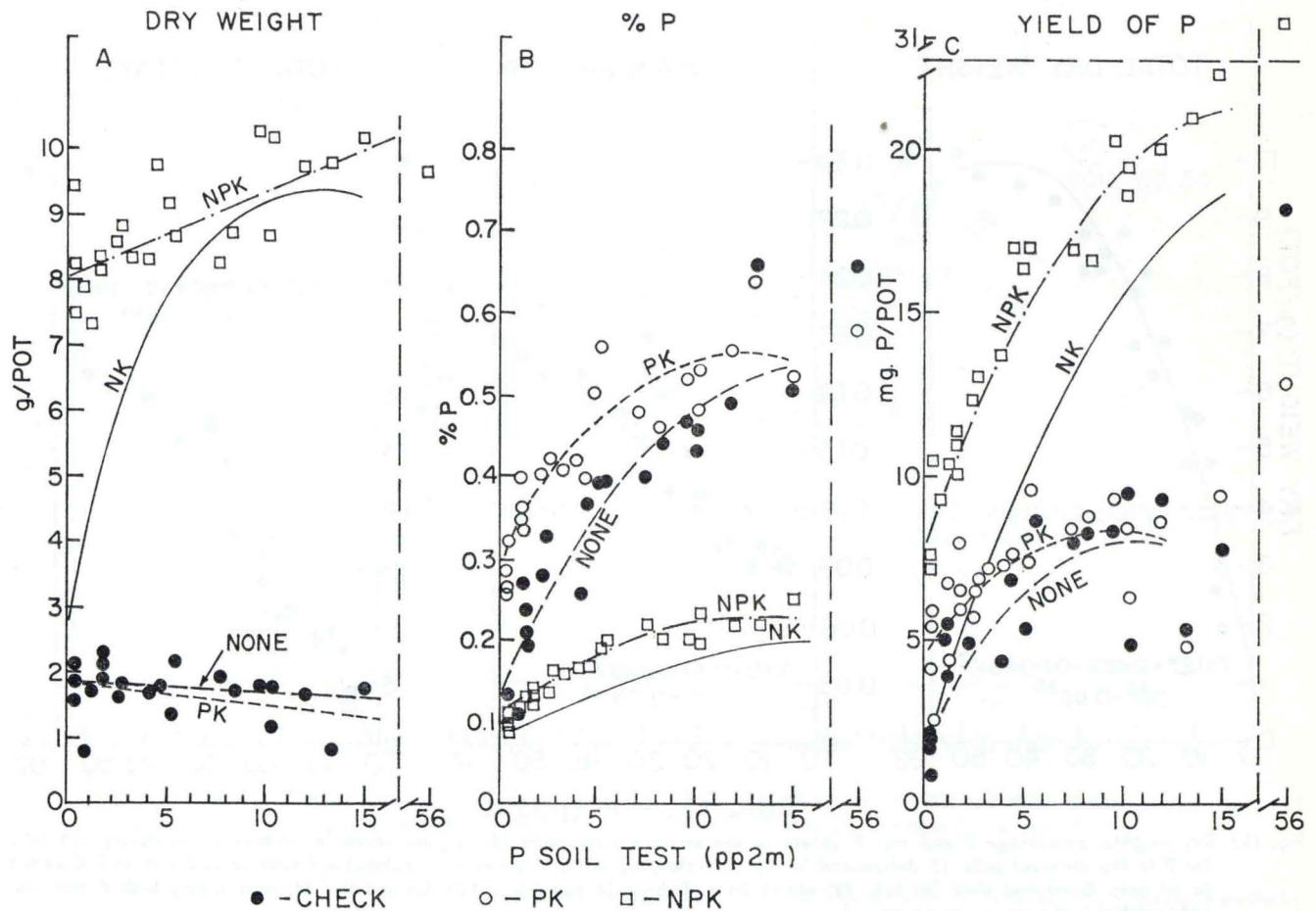


Fig. 14. Relation between the P test values of the soils by the "B" method and the response of ryegrass in dry matter, percentage P and P yield, as affected by the nutrient additions to the sand layer below the soil.

- A. Dry weights.
- B. Percentage P.
- C. P yield (mg. P/pot).

Combined data for Feb. 23, Mar. 23, Apr. 14 and 25, and May 9 harvests.

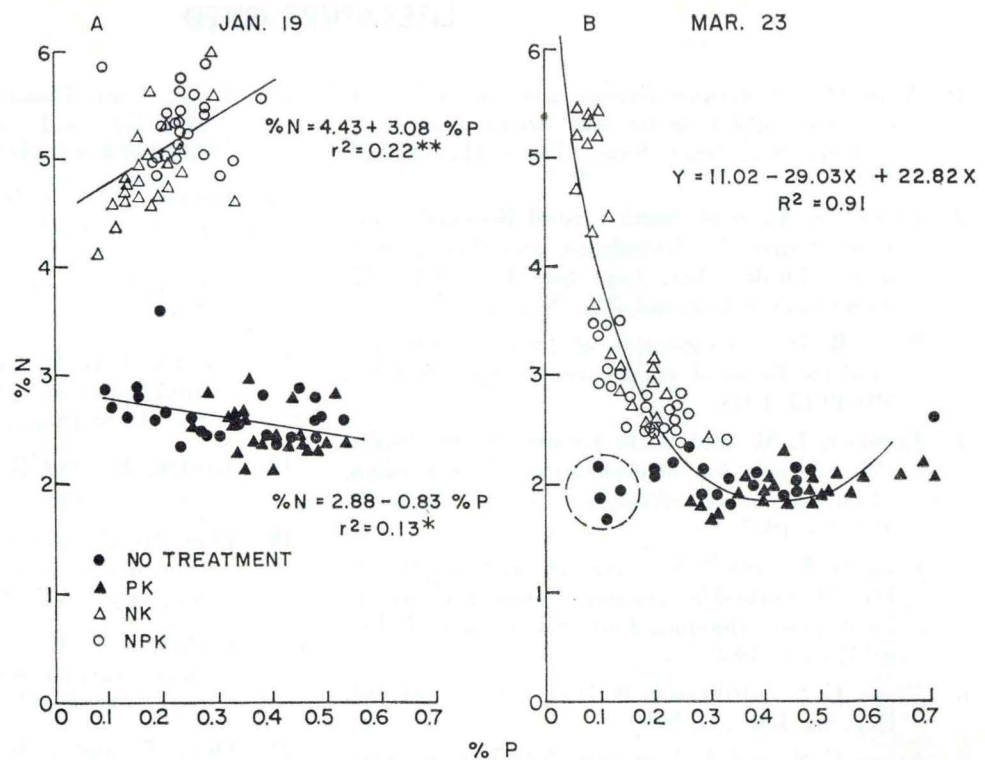
Resume. Data from plant harvests made after the plants had become established in the soils in the greenhouse showed good relationships between the plant uptake of N and P and the laboratory tests for rates of N mineralization and for P availability, respectively. However, the data emphasize that interrelationships between these two nutrients must still be considered—even here where efforts were made to minimize their effects. Only when P availability was considered, as well as the rate of N mineralization in the laboratory, could the laboratory test for N be used effectively to predict N uptake by the plants. Possibly the differences in temperature of testing in the laboratory and greenhouse may be involved in this effect. With the more rapid mineralization of N at the higher temperature in the laboratory, especially for the NH_4^+ production test, a P deficiency in the soil may have reduced the rate of N mineralization to a much greater degree than it did at the lower temperature in the greenhouse. N mineralization at the temperatures used in the laboratory is much accelerated. At 40°C ., NH_4^+ -N production during 7 days in the laboratory averaged over

all soils was 68 pp2m (on a dry-soil basis). At 35°C ., NO_3^- -N production during 14 days in the laboratory averaged 50 pp2m. At 20 - 25°C . in the greenhouse, the ryegrass plants harvested from 110 to 126 days after Jan. 19 contained N equivalent to only 37 pp2m on a dry-soil basis. Additional studies are needed to evaluate this effect of temperature, and possibly other factors, to determine the most satisfactory procedure for testing and interpreting the N test results.

In addition to these P effects upon N, there was a marked effect of N upon P contents of the plants. This interrelation is illustrated in fig. 15. On Jan. 19, there was an inverse relation between percentage N and percentage P in the plants where no N had been added, but a positive relationship where N had been added. Similar relationships were found in the corn plants harvested on that date. This positive relationship has often been observed in samples of corn leaves¹¹ and in bromegrass in

¹¹ L. C. Dumenil. Relationships between the chemical composition of corn leaves and yield responses from nitrogen and phosphorus fertilizer. Unpublished Ph.D. thesis. Iowa State University Library, Ames, Iowa. 1958.

Fig. 15. Relations between percentage N and percentage P in the ryegrass plants on Jan. 19 and on March 23, as influenced by nutrient additions to the sand layer below the soil (circled values are not included in the regression equation).



field experiments (12). Later, on March 23, there was a curvilinear, inverse relationship between percentage N and percentage P in the ryegrass, with the data from all treatments fitting one general relationship. Within each treatment, percentage N and percentage P varied with variations in P availability in the soils. Except for four soils, which were extremely P deficient and to which no N was added, percentage N increased to high levels in the plants as percentage P decreased to very low levels, and percentage P increased to very high levels when percentage N decreased to very low levels. Dry matter yields were low at both extremes.

K contents of the plants were also markedly influenced by the N levels present. In N-deficient plants, percentage K was similar for all levels of soil K. Adding N decreased percentage K in the plants at low levels of soil K but increased percentage K in the plants at high levels of soil K.

Plant uptake of P was highly correlated with P soil-test values even where the plants were extremely N deficient or where P was added to the sand layer below the soil. The data indicate that P added to the sand layer below the soil was taken up by the plant roots and influenced the dry-matter yields of the above-ground plant parts but that the amount of P in the above-ground plant parts was determined by the level of "available" P in the soils. At very low levels of available P in the soils, it appears that some of the P taken up by the plant roots from the sand layer below the soil was lost to the soil, raising the level of extractable P in these soils.

DISCUSSION

The results reported here indicate some of the problems associated with the development of satisfactory relationships between laboratory and greenhouse estimates of nutrient availability in soils. These problems emphasize the importance of using the proper techniques if greenhouse data are to provide satisfactory standards for comparison of different chemical soil tests in the laboratory.

Treatment of the samples before testing can influence nutrient availability in the soils. For example, drying these soils resulted in very different test results for N and K. Other soils may not be influenced in the same way or to the same degree.

The laboratory test results for P and K were markedly influenced by cropping in the greenhouse. In this group of soils, these changes did not destroy the relationships between nutrient uptake by the plants and the initial soil-test levels, but this may not be true for other groups of soils.

The relationships developed here between laboratory and greenhouse indexes of nutrient availability indicate that the techniques used in handling the samples before testing and in conducting the greenhouse experiments appeared to provide good indexes of N and P availability in these soil samples and that the greenhouse indexes may be used as satisfactory standards of comparison of different laboratory tests. The data also indicate that similar greenhouse techniques would provide good standards for comparison of different laboratory tests for K availability in soils.

The best relative indexes of N, P and K availability to plants developed in this study for this group of soils are reported in appendix table A-2.

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APPENDIX

Table A-1. Location of sites from which soil samples were collected.

Soil sample No.	Soil type	Experimental farm	Name of experiment	Crop rotation ^a	Plot sampled
1	Moody sil	Moody	NP	CCO	Check
2	Primghar sil	Galva-Primghar	Legume-Fert. N	COM	Check
3	Galva sil	Galva-Primghar	Rotation-Fertility	Cont. corn	Check
4	Ida sil	Western Iowa	Time & Rate of P	COM	Check
5	Monona sil	Western Iowa	Legume-Fert. N	COM	Check
6	Cresco sil	Howard Co.	Rotation	CCOMM	Check
7	Cresco sil	Howard Co.	Manure PK	COM	Check
8	Moody sil	Moody	Time & Rate of P	COM	Check
9	Cresco sil	Howard Co.	Manure vs N	CCOM	0+30+30
10	Marshall sil	Soil Conservation	Cont. Corn	CCCC	No
11	Belinda sil	Albia	Rotation	Cont. corn	No
12	Edina sil	Southern Iowa	Legume-Fert. N	Cont. corn	Check
13	Grundy sil	Grundy-Shelby	Rock-Super	COM	Check
14	Webster cl	Clarion-Webster	PK (calc)	COM	Check
15	Kenyon sil	Carrington-Clyde	Rotation	CCOMM	Check
16	Seymour sil	Seymour-Shelby	PK	COM	Check
17	Edina sil	Southern Iowa	Rotation-Fertility	CCOM	Check
18	Seymour sil (subsoil)	Seymour-Shelby	Subsoil	COM	Check
19	Webster cl	Agronomy Farm	4-yr. rotation	CCOM	Check (plot 1315)
20	Clarion sil	Agronomy Farm	Cont. Corn	Cont. corn	Check (plot 910)
21	Fayette sil	Linn Co. #15 — Leo Pickerell — Proj. 1377			
22	Downs sil	Linn Co. #32 — L. E. Bingham — Proj. 1377			
23	Tama sil	Muscatine Co. #16 — Don Wildason — Proj. 1377			
24	Tama sil	Muscatine Co. #8 — Ed Brown — Proj. 1377			

^a C = Corn, O = Oats, M = Alfalfa-clover meadow.

Table A-2. Relative indexes of plant availability of N, P and K in the different soil samples based upon the results of cropping with ryegrass in the greenhouse.

Soil No.	N yield ^a		Plant dry weight g./pot	P ^b		K		
	A mg./pot	B mg./pot		Average %P	Yield of P mg./pot	Corn ^c Jan. 1, 1963 %	Grass with corn ^c Jan. 19, 1963 %	Grass ^d May 25, 1963 %
1.....	30.4	28.4	6.80	0.12	8.2	4.96	3.52	2.72
2.....	26.4	28.5	10.19	0.20	20.1	2.03	2.28	2.49
3.....	26.4	26.6	8.46	0.13	10.8	2.43	2.79	2.34
4.....	31.3	22.0	2.93	0.09	2.7	4.48	3.28	2.28
5.....	32.4	29.8	9.81	0.19	18.3	4.82	4.20	2.85
6.....	34.6	31.6	4.75	0.12	5.6	0.76	0.54	1.12
7.....	40.3	30.0	8.12	0.21	16.6	1.42	1.04	2.04
8.....	53.6	37.0	5.60	0.09	5.1	3.00	2.88	2.43
9.....	30.8	29.2	8.11	0.18	14.4	3.33	2.60	2.43
10.....	24.0	18.5	9.40	0.17	15.7	5.66	3.60	2.28
11.....	23.6	31.4	8.76	0.15	13.4	1.52	1.06	1.96
12.....	21.7	27.3	8.77	0.19	16.3	4.16	2.79	2.19
13.....	32.1	35.5	4.81	0.10	4.9	3.38	2.37	2.02
14.....	39.0	30.8	2.99	0.09	2.7	1.52	1.26	1.74
15.....	31.2	30.2	6.30	0.10	6.4	1.44	0.98	1.80
16.....	29.2	30.2	5.50	0.10	5.6	1.64	1.98	1.89
17.....	23.3	35.1	8.03	0.13	10.6	1.52	0.74	1.60
18.....	23.6	13.8	9.52	0.19	18.0	5.03	4.50	2.70
19.....	32.4	27.8	2.52	0.09	2.3	1.41	0.90	1.40
20.....	19.8	8.9	1.93	0.09	1.9	1.29	1.02	1.77
21.....	32.1	27.9	—	—	—	—	—	—
22.....	28.8	30.6	6.86	0.10	7.0	2.23	1.24	2.22
23.....	23.1	21.4	7.74	0.13	9.7	2.60	1.62	2.20
24.....	28.4	41.0	10.15	0.30	29.9	6.03	4.11	2.70

^a Average of check and PK treatments. A=N taken up by corn and grass from Dec. 2, 1962, to Jan. 19, 1963, for estimating readily available NO₃-N in soil initially. B=N taken up by grass from Jan. 19, 1963, to May 9-25, 1963, for estimating N released from unavailable to available forms over time.

^b NK treatment, grass. Jan. 19, 1963, to May 14, 1963.

^c Check treatment.

^d NP treatment.