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Variations in the Natural Abundance of ^{15}N of Wheat Plants in Relation to Fertilizer Nitrogen Applications[1]

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ABSTRACT

Measurements were made by two laboratories of the $\delta^{15}N$ (per mill ^{15}N excess) of winter wheat plants (*Triticum aestivum* L.) grown at five locations in Pennsylvania on experimental plots. The plots were fertilized with N at various rates. The results from both laboratories showed a consistent decline in $\delta^{15}N$ with increasing rates of N application. Such a decrease in $\delta^{15}N$ is consistent with increasing contributions of fertilizer N to the plants as the rate of application increased, given that fertilizer N has a lower ^{15}N content than the soil N. The coefficients of regression of $\delta^{15}N$ of wheat on N application rate were always negative and usually significantly different from zero. The regression coefficients computed from the results of the two laboratories were not significantly different from each other in 12 of 16 experiments. There was, however, a systematic, unexplained difference in the results from the two laboratories. A regression of one set of data on the other resulted in a regression coefficient significantly different from one, the theoretically expected value.

Additional Index Words: $\delta^{15}N$, $^{15}N/^{14}N$, N isotope ratios.

A METHOD, based upon variations in the natural abundance of ^{15}N, was proposed by Kohl et al. (6) for estimating the fractional contribution of fertilizer nitrogen to nitrate produced by soils. This method depends, in part, on a measurable difference in the ^{15}N content of the major sources of the nitrate produced by the soil. Subsequent work (9, 3) showed that the ^{15}N content of a number of fertilizer samples was significantly lower than that of nitrate produced by several central Illinois soils. Therefore, provided that these two sources are the major ones, and that analytical errors and errors introduced through isotopic alteration of the nitrate sources by isotopic fractionation are not too great, it should be possible to detect fertilizer-derived N by measuring the ^{15}N content of nitrate produced by these soils. Similarly, fertilizer N in the total N of plants grown on these soils should also be detectable by the isotopic composition of the total N in the plant tissue.

Kohl et al. (7) showed that the ^{15}N composition of the total N of grain and leaf samples of corn (*Zea mays* L.) decreased systematically as N fertilizer rates increased. This result is consistent with increasing contributions of fertilizer N to the plants as the rate of N application increased. In a greenhouse experiment, the total N of sudangrass [*Sorghum sudanense* (Piper) Stapf] grown on several central Illinois soils and fertilized with unlabeled $(NH_4)_2SO_4$ was consistently lower in ^{15}N than was the N of unfertilized plants (8). In the laboratory, the ^{15}N composition of nitrate produced by these same soils usually was significantly lower when the soils were incubated in the presence of unlabeled $(NH_4)_2SO_4$ than when incubated in the absence of fertilizer (4). From the results of these experiments, it appears that the presence of fertilizer N in plants grown on these soils and in nitrate produced from them may be detected by measurements of the natural abundance of ^{15}N.[3]

However, data reported by other workers indicate that it may not always be possible to detect fertilizer N by measuring the natural abundance of ^{15}N. Bremner and Tabatabai (1) found that the ^{15}N content of the total N of a variety of soils and the nitrate produced from them was considerably lower than that of the central Illinois soils (3). Furthermore, the ^{15}N content of the total N of the soils and of the nitrate produced from them varied more widely in these soils than in the central Illinois soils (3, 9). Although these workers did not report ^{15}N measurements on fertilizer N, it does not seem likely, in view of the low ^{15}N

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[3] Two of these same experiments showed that estimates, based on natural ^{15}N abundance, of the fractional contribution of fertilizer N to the nitrate or to the plants were not as precise as were estimates based on ^{15}N-enriched fertilizer. This result is expected, since the difference in ^{15}N content of the fertilizer and soil-derived nitrate—that is, the difference on which the estimate is based—is much greater for the ^{15}N-enriched fertilizer. The estimates based on natural abundance of ^{15}N were also not as accurate as those based on the ^{15}N-enriched method, since they frequently underestimated, but never overestimated, the fractional contribution of fertilizer N to plants or to nitrate produced by the soils.

content of these soils and the variability in ^{15}N content among them, that they would have found measurable differences in ^{15}N content between soil-derived nitrate and fertilizer N. Hence, it would not be possible from measurements of the natural ^{15}N abundance to detect the presence of fertilizer N in nitrate produced from or in plants grown on those soils. Edwards (2) was unable to measure significant differences between the ^{15}N content of nitrate produced by fertilized and unfertilized samples of one of the soil types used in the Bremner and Tabatabai (1) study.

The discrepancy between the results with central Illinois soil (3, 4, 7, 8) and those reported by Bremner and Tabatabai (1) and Edwards (2) may have resulted from intrinsic differences in the soils used or from differences in analytical procedure or instrumentation. If use of variations in the natural abundance of ^{15}N is to be pursued as a generally applicable tool for studies of the N-cycle, it should be ascertained whether results comparable to those obtained with central Illinois soils can be obtained with soils in other areas. It is also important to determine whether different laboratories can obtain comparable results.

This paper describes the results of ^{15}N measurements of the total N of field-grown winter wheat plants (*Triticum aestivum* L.) fertilized with N applied at different rates. The experiment was conducted by A. S. Hunter, Professor of Soil Fertility, Agronomy Department, The Pennsylvania State University and G. Stanford, Soil Scientist, US Soils Laboratory, Beltsville, Maryland, to determine the N requirement of winter wheat (10), soil N availability in the field, and the protein content of winter wheat in relation to the rate and time of N application (5). These investigators supplied the samples for the present study. The objectives of the work described here were: (i) to determine whether the negative relationship between ^{15}N of the total N of a crop and the rate of N application observed in corn grown in central Illinois (7) existed with another crop grown at different locations; and (ii) to establish whether the same samples would yield similar results when analyzed independently in two different laboratories.

METHODS AND MATERIALS

Field experiments were conducted with fall-seeded wheat in 1970–71 at five locations in Pennsylvania. Table 1 shows the soil type at each location. Experimental details have been reported (10). Briefly, the wheat was fertilized with 0, 34, 67, 101, 135, and 168 kg N/ha as $(NH_4)_2SO_4$. The fertilizer was applied in the spring or the fall at all locations and as a divided application at one location (Centre County). At two locations (Centre County and Lancaster County) the wheat varieties, 'Blueboy' and 'Redcoat,' were grown. At the other three locations only Blueboy was grown. There were four replicates for each treatment. In July 1971, whole plants were harvested from two positions in each plot and composited. The plant samples were dried at 65C and ground in a Christie-Norris Mill to pass a 0.5-mm screen. Subsamples were analyzed at the Center for the Biology of Natural Systems (CBNS), Washington University, St. Louis, Missouri and at the Plant Nutrition Laboratory, Agricultural Research Service, U.S. Department of Agriculture, (USDA) Beltsville, Maryland.

Table 1—Soil types at the locations of the experiments

Location	Soil type
Agronomy Research Farm Centre County, Pa.	Hublersburg silt loam
S. E. Field Research Station Lancaster County, Pa.	Hagerstown silt loam
Cumberland County, Pa.	Hagerstown silt loam
Franklin County, Pa.	Duffield silt loam
Snyder county, Pa.	Allenwood silt loam

At the CBNS laboratory, all samples were subjected to micro-Kjeldahl digestion without exogenous reducing agents. The digests were steam distilled and prepared for mass spectrometric analysis as described previously (9). The mass spectrometer used was a Consolidated-Nier 21-201. At the USDA laboratory, samples were analyzed only from plots fertilized with 0, 101, and 168 kg N/ha and reducing agents (salicylic acid and thiosulfate) were included in the macro-Kjeldahl digestions. Except for the difference between the two laboratories in the method of Kjeldahl digestion, the samples were prepared in the same manner for mass spectrometric analysis. The mass spectrometer used in the USDA laboratory is an AEI MS 20.

The unit of measure for the ^{15}N content used in this paper is the per mill ^{15}N enrichment ($\delta^{15}N$) compared to a reference standard:

$$\delta^{15}N = \frac{\text{atom \% } ^{15}N \text{ (sample)} - \text{atom \% } ^{15}N \text{ (standard)}}{\text{atom \% } ^{15}N \text{ (standard)}} \times 1000.$$

The reference standard used in the CBNS laboratory was $(NH_4)_2SO_4$. This standard was 1.4 ± 0.7 $\delta^{15}N$ units compared to atmospheric N. The reference standard used in the USDA laboratory was an ultra-high-purity compressed N_2 gas which was -6.5 $\delta^{15}N$ units with respect to the CBNS standard, or -5.1 $\delta^{15}N$ units compared to atmospheric N.

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show $\delta^{15}N$ values of the total N of wheat plants as a function of the application rate of fertilizer N at the five locations in Pennsylvania. $\delta^{15}N$ values for individual replicates are shown. The filled circles represent two or more replicate samples which have the same $\delta^{15}N$ value. The lines which appear in each panel represent linear regressions through the experimental points. The correlation coefficients and the probability that the correlations are not significantly different from zero are shown in each panel. The choice of the linear form for the regressions was based on data obtained by Stanford and Hunter (10) from the same samples used in the present study. These data showed a linear relationship between the rate of N application and plant uptake of N. However, a curvilinear relationship between $\delta^{15}N$ of the plant N and the rate of N application cannot be ruled out by the data presented in this paper. The variability in ^{15}N content of plants taken from replicate plots is too large to allow a distinction between a linear and curvilinear relationship.

The correlations between the two parameters, $\delta^{15}N$ of the total N of the wheat plants and the rate of application of N fertilizer, were all negative. The correlation coefficients usually were significant ($p < 0.05$). Of 18 experiments at five locations, 16 showed a significant negative correlation between the two parameters when the regressions were based on analyses done in the CBNS laboratory and 12 were significant when the regressions were based on the USDA results. (The difference between the two laboratories in the number of experiments in which significant correlations

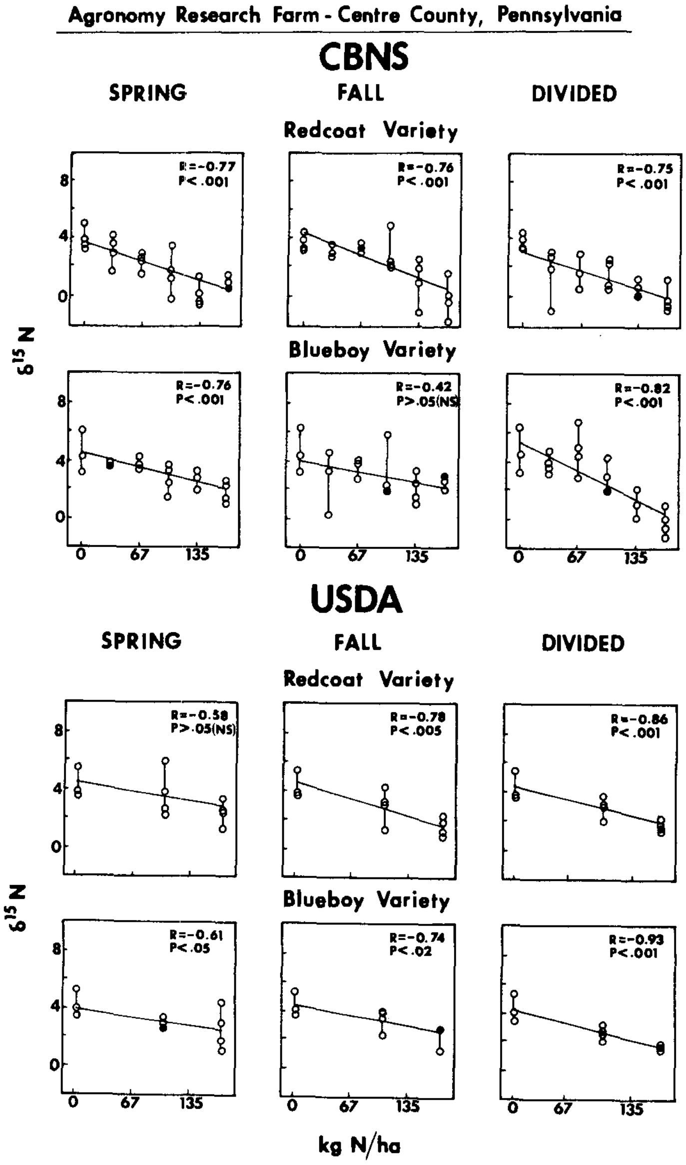

Fig. 1—The $\delta^{15}N$ of the total N of two varieties of wheat plants (as analyzed at the CBNS and USDA laboratories) as a function of the application rate of fertilizer N applied in the spring or fall or as a divided application to experimental plots in Centre County, Pennsylvania.

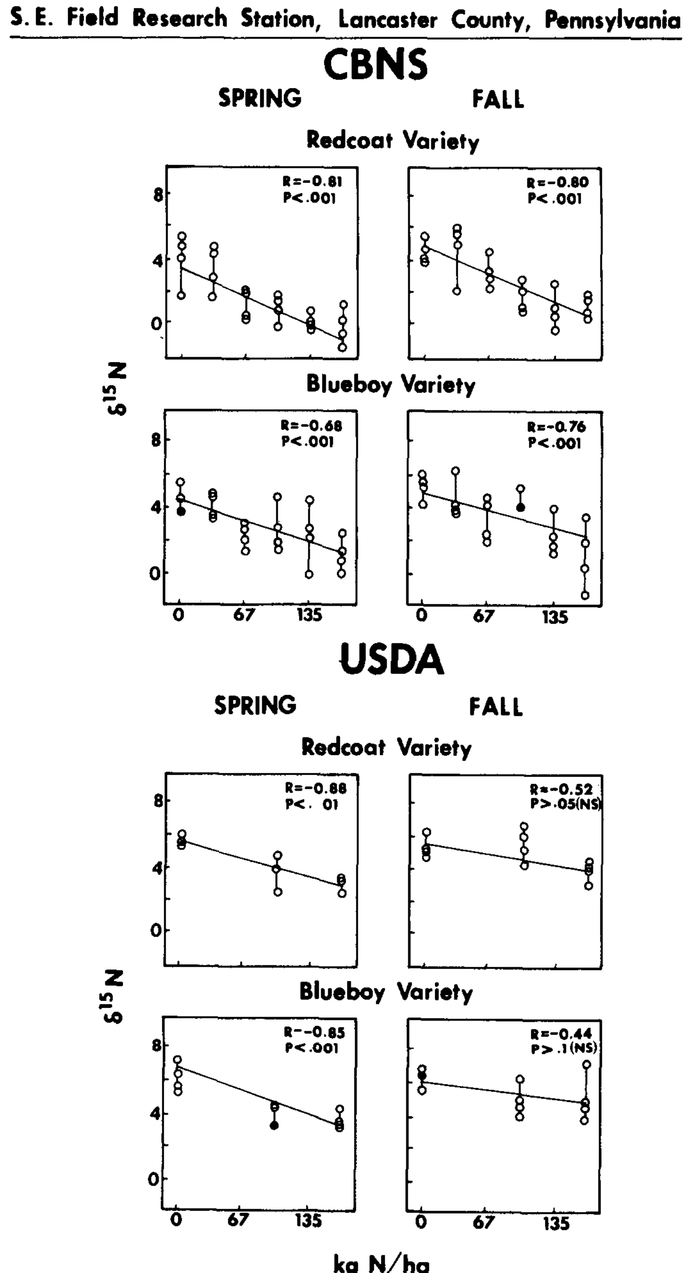

Fig. 2—$\delta^{15}N$ of the total N of two varieties of wheat plants (as analyzed at the USDA and CBNS laboratories) as a function of the application rate of fertilizer N applied in the spring or fall to experimental plots in Lancaster County, Pennsylvania.

were observed may have been due to fewer samples being analyzed by the USDA laboratory.)

The negative correlations between $\delta^{15}N$ of the total N of the wheat plants and the rate of N application are consistent with increasing contributions of fertilizer N to the total N of the plant as the rate of application increased, provided that the $\delta^{15}N$ of the fertilizer N was lower than that of the soil-derived N. The $(NH_4)_2SO_4$ used as fertilizer in these experiments was not available for ^{15}N analysis. However, on the basis of a previously published survey of the isotopic composition of ammonium-containing fertilizers (9) the ^{15}N content may be assumed to be low relative to that of the N supplied by the soils used in this study, as judged by the $\delta^{15}N$ value of the unfertilized wheat samples.

Table 2 gives linear regression equations for the relationship between $\delta 1^5N$ values of the wheat (Y) and the rate of application of fertilizer N (X, kg N/ha). As can be seen in Fig. 1 to 3 and in the regression equations shown in Table 2, the same qualitative conclusion may be drawn from results of analyses done in the two laboratories: that increasing the rates of N application lowered the $\delta^{15}N$ of the total N of the plants. All regression coefficients were negative. The slopes usually were significantly different from zero at the 0.025 level. The range in values for the regression coefficients differed somewhat for the two sets of data. The regression coefficients of the USDA regressions were, with three exceptions, lower than those of the corresponding CBNS regressions. An analysis of covariance was done to test whether the differences in regression coef-

Table 2—A comparison between CBNS and USDA linear regression equations for the relationship between $\delta^{15}N$ of wheat plants (Y) and the rate of N application (X, kg/ha) and correlation coefficients (R)

Location	Time of N application	Variety	n	Regression equation based on CBNS results†	R	n	Regression equation based on USDA results†	R
Centre Co.	Spring	Redcoat	24	Y = 3.7(±0.5) - 0.0184(±0.0079)X	-0.77*	11	Y = 4.4(±1.0) - 0.0113(±0.0144)X	-0.58
	Fall	Redcoat	24	Y = 4.5(±0.6) - 0.0233(±0.0102)X	-0.76*	11	Y = 4.3(±0.8) - 0.0158(±0.0115)X	-0.78*
	Divided	Redcoat	24	Y = 3.2(±0.5) - 0.0197(±0.0089)X	-0.75*	11	Y = 4.3(±0.6) - 0.0152(±0.0089)X	-0.86*
	Spring	Blueboy	21	Y = 4.5(±0.4) - 0.0153(±0.0072)X	-0.76*	11	Y = 4.1(±0.8) - 0.0103(±0.0121)X	-0.61*
	Fall	Blueboy	21	Y = 3.9(±0.7) - 0.0103(±0.0128)X	-0.42	10	Y = 4.4(±0.7) - 0.0116(±0.0108)X	-0.74*
	Divided	Blueboy	23	Y = 5.2(±0.6) - 0.0296(±0.0105)X	-0.80*	11	Y = 4.2(±0.4) - 0.0115(±0.0057)X	-0.93*
Lancaster Co.	Spring	Redcoat	24	Y = 3.7(±0.6) - 0.0259(±0.0098)X	-0.81*	10	Y = 5.9(±0.6) - 0.0162(±0.0082)X	-0.88*
	Fall	Redcoat	24	Y = 5.0(±0.6) - 0.0255(±0.0098)X	-0.80*	12	Y = 6.1(±0.8) - 0.0072(±0.0121)X	-0.52
	Spring	Blueboy	24	Y = 4.5(±0.6) - 0.0196(±0.0110)X	-0.68*	12	Y = 7.7(±0.6) - 0.0159(±0.0083)X	-0.85*
	Fall	Blueboy	24	Y = 3.9(±0.6) - 0.0262(±0.0160)X	-0.76*	12	Y = 6.9(±0.7) - 0.0076(±0.0102)X	-0.44
Cumberland Co.	Spring	Blueboy	24	Y = 5.8(±0.6) - 0.0242(±0.0075)X	-0.75*	12	Y = 6.1(±0.8) - 0.0241(±0.0113)X	-0.87*
	Fall	Blueboy	24	Y = 3.6(±0.5) - 0.0139(±0.0094)X	-0.61*	12	Y = 6.8(±0.7) - 0.0231(±0.0096)X	-0.89*
Franklin Co.	Spring	Blueboy	24	Y = 5.2(±1.0) - 0.0261(±0.0170)X	-0.62*	12	Y = 4.8(±0.7) - 0.0149(±0.0096)X	-0.79*
	Fall	Blueboy	24	Y = 6.4(±1.0) - 0.0217(±0.0168)X	-0.57*	12	Y = 5.0(±0.7) - 0.0164(±0.0098)X	-0.81*
Snyder Co.	Spring	Blueboy	24	Y = 4.0(±0.6) - 0.0116(±0.0105)X	-0.50*	12	Y = 4.3(±0.9) - 0.0045(±0.0132)X	-0.27
	Fall	Blueboy	23	Y = 3.7(±0.8) - 0.0066(±0.0132)X	-0.25	12	Y = 4.2(±1.3) - 0.0041(±0.0182)X	-0.19

* Significantly different from zero ($p < 0.05$).
† Error terms represent the 95% confidence interval.

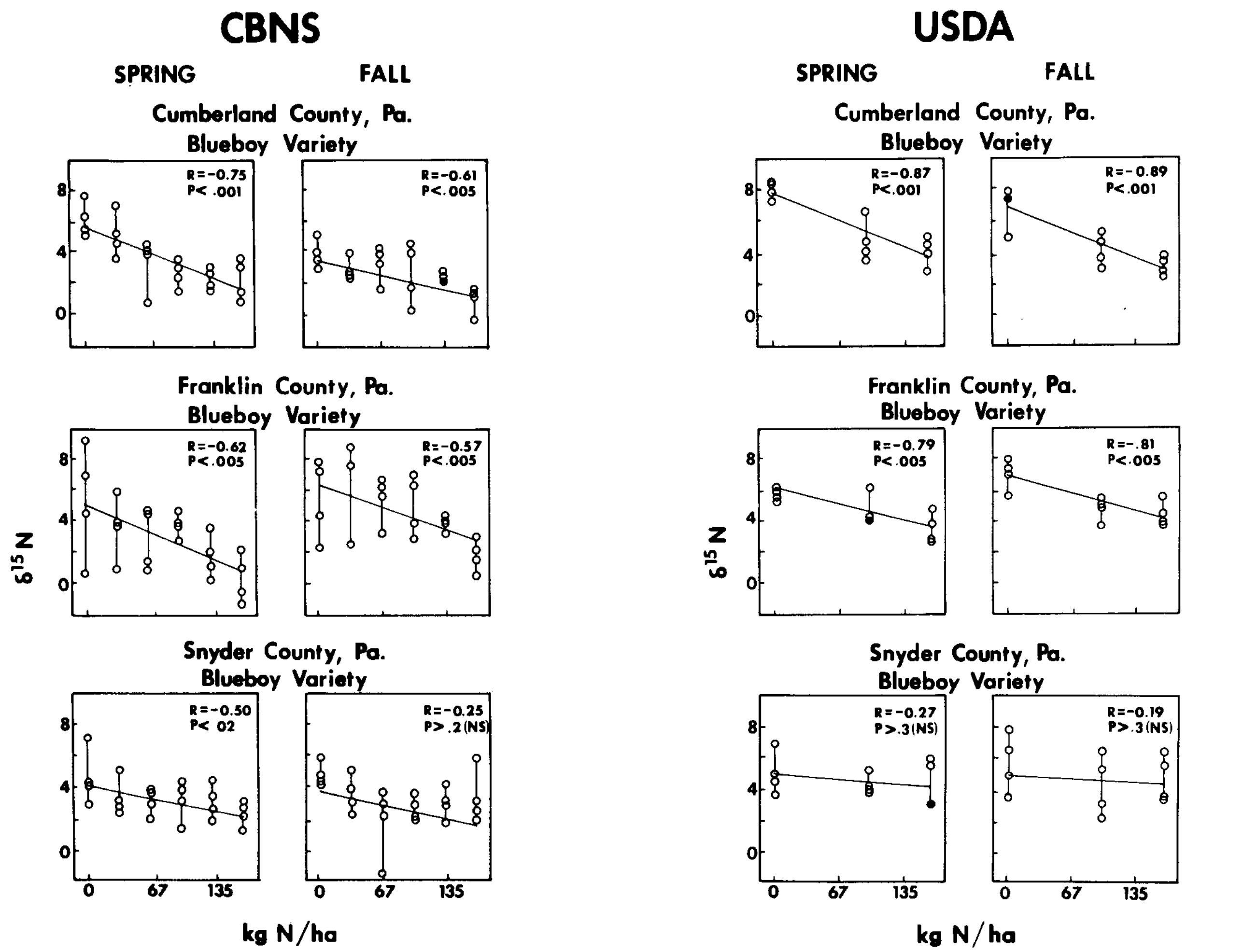

Fig. 3—The $\delta^{15}N$ of the total N of wheat plants (as analyzed at the CBNS and USDA laboratories) as a function of the application rate of fertilizer N applied in the spring or fall to experimental plots at three locations in Pennsylvania.

ficients computed from USDA vs CBNS results are significant. With respect to the slope of the regression of $\delta^{15}N$ of wheat on the N application rate, the results of analyses made in the two laboratories did not differ significantly from each other, with four exceptions (Table 3). In three of these exceptions, the regression coefficients computed from the CBNS data had significantly larger negative slopes than those computed from the USDA data. In the fourth, the experiment in which N was applied in the fall to plots in Snyder County, the individual regression coefficients were not significantly different from zero, nor was the common regression coefficient significantly different when computed from the pooled data.

The tests for homogeneity of regression coefficients com-

Table 3—CBNS vs. USDA results. Test for homogeneity of regression coefficients for $\delta^{15}N$ of wheat on rate of N application (kg/ha) computed for CBNS vs. USDA data and common regression coefficients for combined data

Location	Time of N application	Variety	df_n†	df_d†	F‡	Common regression coefficient
Centre Co.	Spring	Redcoat	1	31	0.99	- 0.0158 ± 0.0067
	Fall	Redcoat	1	31	1.36	- 0.0206 ± 0.0075
	Divided	Redcoat	1	31	0.68	- 0.0180 ± 0.0062
	Spring	Blueboy	1	28	0.18	- 0.0133 ± 0.0061
	Fall	Blueboy	1	27	0.01	- 0.0111 ± 0.0086
	Divided	Blueboy	1	30	8.06*	- 0.0228 ± 0.0078
Lancaster Co.	Spring	Redcoat	1	32	2.87	- 0.0219 ± 0.0068
	Fall	Redcoat	1	32	8.60*	- 0.0178 ± 0.0081
	Spring	Blueboy	1	30	0.31	- 0.0182 ± 0.0075
	Fall	Blueboy	1	32	7.47*	- 0.0184 ± 0.0087
Cumberland Co.	Spring	Blueboy	1	32	0.34	- 0.0242 ± 0.0077
	Fall	Blueboy	1	32	2.59	- 0.0178 ± 0.0068
Franklin Co.	Spring	Blueboy	1	32	1.40	- 0.0214 ± 0.0111
	Fall	Blueboy	1	31	0.34	- 0.0195 ± 0.0106
Snyder Co.	Spring	Blueboy	1	32	1.10	- 0.0087 ± 0.0078
	Fall	Blueboy	1	32	10.4*	- 0.0056 ± 0.0100

* Regression coefficients derived from CBNS and USDA results are significantly different at the 0.05 level.
† df_n refers to the degrees of freedom in the numerator and df_d refers to the degrees of freedom in the denominator.
‡ F is derived from an analysis of covariance as described on p. 319, Steel and Torrie (11). Error term for common regression coefficient represents the 95% confidence interval.

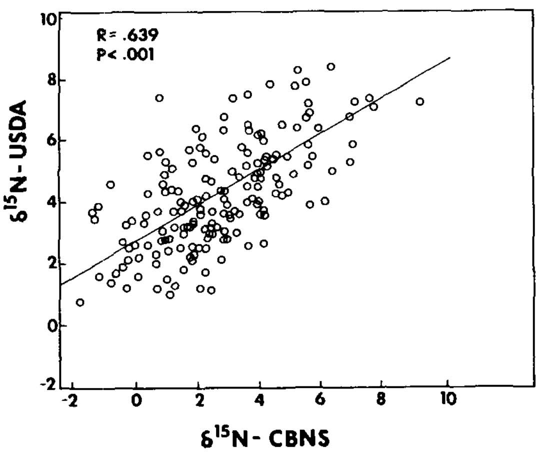

Fig. 4—$\delta^{15}N$ of the total N of wheat plants as analyzed at the USDA laboratory plotted against $\delta^{15}N$ of the same samples as analyzed at the CBNS laboratory.

pare the entire body of data from each experiment as analyzed by the two laboratories. A one-to-one sample comparison is more direct. Figure 4 shows a plot of the CBNS data against the USDA data. The correlation coefficient for the relationship between the two data sets of 0.639 indicates that there is considerable unexplained variation. Nevertheless, the correlation is highly significant ($p < 0.001$). The equation for the regression is:

$$\delta^{15}N_{USDA} = 2.76\ (\pm\ 0.23) + 0.530\ (\pm\ 0.111)\ \delta^{15}N_{CBNS};^4$$

as compared with the theoretical expectation:

$$\delta^{15}N_{USDA} = 6.5^5 + \delta^{15}N_{CBNS}.$$

If the four experiments that showed significant differences between the two data sets in the regression coefficients for the relationship between the $\delta^{15}N$ of wheat plants and the N application rate are dropped, the experimental regression equation for the relationship between the CBNS and USDA data is essentially unchanged:

$$\delta^{15}N_{USDA} = 2.58\ (\pm\ 0.25) + 0.561\ (\pm\ 0.122)\ \delta^{15}N_{CBNS}.$$

We cannot explain why a given change in $\delta^{15}N_{CBNS}$ is accompanied by a change just over half as great in $\delta^{15}N_{USDA}$, other than to suggest that it is caused either by a difference in the response of the two mass spectrometer outputs as the ^{15}N content varies or to a difference in sample preparation in the two laboratories.

CONCLUSIONS

In both laboratories, the results showed that the $\delta^{15}N$ of wheat plants decreased as the N application rate increased. The regression coefficients for the relationship between the two parameters were always negative and significantly different from zero in most cases. This decrease in $\delta^{15}N$ of wheat with increasing N application rates is consistent with increasing contributions of fertilizer N to the plants, given that fertilizer N has a lower ^{15}N content than soil N. The regression coefficients computed from the results of analyses by the two laboratories were not significantly different from each other in most cases. The values for $\delta^{15}N$ of wheat plants obtained at the two laboratories were significantly correlated. However, a regression of USDA data on CBNS data had a regression coefficient of 0.53 rather than 1.0, the theoretically expected value.

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[4] Error terms represent the 95% confidence interval.
[5] The theoretically expected intercept is 6.5 because of the difference in ^{15}N content of the reference standards used in the two laboratories as mentioned above.

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