

# IOWA EXPERIENCE WITH THE ILLINOIS N TEST<sup>1</sup>

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## Introduction

An important aspect for estimating optimal N applications is to predict the soil N supply capacity (ability to supply plant-available N) each year. Since fertilizer applications supplement crop N requirements that the soil system cannot meet, understanding potential plant-available N originating from the soil can aid in setting N fertilization rates. General differences in plant-available N and fertilization need between fields are commonly accounted for through effects of crop rotation. An example of further refinement is the grouping of similar N responsive soils by yield potential (Vanotti and Bundy, 1994), as is done in Wisconsin for corn N fertilization. Determining soil N supply (N responsiveness) through soil testing has received considerable attention over the years. Most commonly used tests measure soil nitrate, either nitrate already present in the soil (post-harvest profile nitrate or preplant profile nitrate - PPNT) or a combination of nitrate present in the soil and that mineralized in the early spring (presidedress soil nitrate - PSNT or late spring soil nitrate - LSNT). For various reasons (time of sampling, sample depth, sampling difficulties, reliability) these tests have not been widely used in the north-central USA (Fox et al., 1999). Many other soil tests, based on various chemical extraction or biological incubation methods, have been studied over the years as potential indicators of mineralizable-N (examples in Bundy and Meisinger, 1994). However they have either not been successful in quantifying potential plant-available N or are not suitable for routine analysis.

Many studies have shown corn response to applied N varies by site and season (examples in Bundy and Malone, 1988; Blackmer et al., 1989; Brown et al., 1993; Schmitt and Randall, 1994; Killorn et al., 1995), with influences from several factors such as weather, soil N processing, N application history, and crop management. Yearly yield with fertilized corn has not been well correlated to optimum N. This indicates that soil supply and climatic factors are more important in determining corn N fertilization needs (Vanotti and Bundy, 1994). Research conducted in Iowa by Killorn et al. (1995), indicated many sites in some years did not respond to applied N, but others had significant response. Similar results have been documented in other studies. Although it is difficult to build plant-available inorganic-N in soils (because of the ease for nitrate losses), there appears to be the potential to build a labile N source (most likely an organic fraction) as a result of either fertilizer or manure application. This source can impact soil supply of plant-available N. Research in Wisconsin by Motavalli et al. (1992) documented that a long-term high fertilizer N rate history influenced plant-available N and corn response to applied N for several years, even after soil profile nitrate had been depleted.

Two important conclusions were stated in the summary of the Killorn et al. (1995) research: one, development of a N soil test is needed that can identify nonresponsive fields; and two, development of a N soil test is needed where samples can be collected before planting. Having the ability to sample well before planting brings a soil N testing option to producers who

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are not willing to wait for spring sampling or do not sidedress N and are not willing to adopt in-season soil sampling. The need exists for an improved soil test to predict field responsiveness to applied N, especially for differentiating nonresponsive and low-responsive sites from responsive sites (situations where greatest economic and environmental improvement could occur). It would be important for this test to have flexible timing, use routine sampling and handling methods, be adaptable for routine laboratory procedures, and to work across multiple soils, yield levels and management systems.

### Basis for the Illinois Soil N Test

Study of organic soil N forms has often utilized a chemical fractionation procedure based on liberation of N compounds by heating soil with strong acid for 12 to 24 hours (Stevenson, 1996). This process is called acid hydrolysis. The N forms are separated by various analysis procedures into the following fractions: acid insoluble-N,  $\text{NH}_3$ -N, amino acid-N, amino sugar-N, and hydrolyzable unknown-N. The main organic N compounds that can be identified are the amino acids and amino sugars. Recent research by University of Illinois researchers (Mulvaney and Khan, 2001) documented a flaw in the conventional steam distillation procedure used for determining the amino acid and amino sugar fractions in the acid hydrolysate. In particular, the amino-sugar fraction has been underestimated in past fractionation work. This has likely caused an underestimation of the effects of cropping, tillage, rotation, and N management on the amino sugar level in soils. Especially pertinent to predicting crop responsiveness to applied N, the hydrolyzable amino sugar-N fraction has not previously been shown to be sensitive to these differences. Hence, the amino sugar fraction has not been well correlated to crop N responsiveness or predictive of nonresponse.

Amino sugars in soils are generally assumed to be of microbial origin. They occur as structural components of mucopolysaccharides and in combination with other compounds. Studies indicate many potential amino sugar compounds in soil, but most occur as glucosamine and galactosamine. While the amino sugar-N fraction is a labile source of soil N, it should be more stable than an inorganic form such as nitrate.

Mulvaney and Khan (2001) developed a diffusion procedure for analysis of soil hydrolysates that improves accuracy and specificity in determination of the amino sugar and other organic N fractions. Using soil samples (mid-March to mid-April, 0-12 inch depth before planting) and corn yield response data from 18 previous field research sites in Illinois (Brown, 1996), Mulvaney et al. (2001) found that the newly developed diffusion procedures resulted in the amino sugar-N fraction being predictive if a site would respond to applied N (correctly categorized all tested sites as either responsive or nonresponsive – most importantly those that previously could not be by other tests). They also found the amino sugar-N fraction was related to the relative magnitude of corn yield increase to applied N at responsive sites. Sites were classified as responsive if the amino sugar concentration was  $<200 \text{ mg kg}^{-1}$ , and nonresponsive at  $>250 \text{ mg kg}^{-1}$  (Mulvaney et al., 2001). Since the acid hydrolysis and fractionation procedure requires at least 12 hours of complicated laboratory work, Khan et al. (2001) developed a simple and rapid soil test procedure designed for use in routine laboratory analysis (the Illinois Soil N Test). The Illinois Soil N Test measures N as ammonia liberated from organic N compounds (expected to be amino sugar-N) and exchangeable ammonium-N during direct soil diffusion for five hours at 48-50°C with 2 M sodium hydroxide (NaOH). The test does not measure nitrate-N. Since the procedure includes exchangeable ammonium-N, soil samples should not be collected after recent application of ammonium containing fertilizers, manure, or sewage sludge. If it is necessary to run the Illinois Soil N Test in those situations, then exchangeable ammonium-N could be determined and the soil test value corrected for the ammonium-N. Development work

by Khan et al. (2001) indicated correct classification of 25 Illinois soils (0-12 inch depth) from the work of Brown (1996) as responsive if the Illinois Soil N Test was  $<225 \text{ mg kg}^{-1}$  and nonresponsive if the test was  $>235 \text{ mg kg}^{-1}$ . Test results are expected to be higher with 0-6 inch depth samples.

### The Illinois Soil N Test Procedure

Details of the method and materials required to perform the test can be found in Khan et al. (2001). Following is the general procedure taken from that publication. This is also the procedure used in our laboratory. A 1.00 g sample of air-dried soil (ground to pass  $< 2\text{mm}$ ) is weighed and carefully placed to the bottom of a 1-pint Ball<sup>®</sup> Mason jar. A petri dish is suspended from a Mason jar lid, and 5 ml of 4% boric acid ( $\text{H}_3\text{BO}_3$ )-indicator solution is dispensed into the petri dish. Ten mL of 2 M NaOH is added to the soil in the Mason jar, and taking care to minimize soil splashing onto the jar wall, the contents are gently stirred until the entire soil sample is wetted. Within 15 to 30 seconds the lid is placed on the jar and sealed with a screw band. The sealed jar is transferred to a hot plate or griddle and heated for precisely 5 hours at 48-50°C (the temperature of the hot plate is set by placing an open Mason jar in the middle of the hot plate that contains 100 ml of distilled water with a thermometer immersed in the water). The jars are gently swirled and moved to a different position on the hot plate once approximately half way through the 5 hour heating. A maximum of 8 jars are placed in the middle portion of each hot plate, and when moved they occupy an inside or outside position (where another jar had been located) for the same length of time. After 5 hours the jars are removed from the hot plate, allowed to cool, opened, and the petri dish removed from the jar lid. Five ml of distilled water is added to the boric acid-indicator solution in the petri dish, and then the amount of diffused N trapped in the boric acid-indicator solution is determined by titration with standard 0.01 M sulfuric acid ( $\text{H}_2\text{SO}_4$ ) acid. Prior to titration of samples, the pH titration endpoint is set with a solution of 5 ml boric acid-indicator solution plus 5 ml distilled water. Test values are not adjusted for a non-soil blank. After diffusion Mason jars are rinsed with hot tap water, rinsed in dilute sulfuric acid, rinsed with hot tap water, and rinsed with distilled water. Petri dishes are rinsed with hot tap water, rinsed with distilled water, soaked overnight in distilled water, and rinsed with distilled water. Mason jar lids are rinsed with hot tap water and rinsed with distilled water.

The Illinois Soil N Test is a non-terminating procedure. That is, the test process does not measure a finite amount of diffusible soil N. The diffusion process, and resultant amount of liberated N, is stopped by removal of the Mason jar from the heating plate and removal of the petri dish (boric acid indicator solution) from the jar. This also means that test results can be influenced by conditions encountered during the procedure – including temperature of the heat source, variability in temperature across the heat source, interface between the heat source and the Mason jar and other jars, the length of heating, and strength of NaOH (Khan et al., 2001). Therefore the test should be run with adherence to conditions used in the original test development and calibration.

### Preliminary Research in Iowa

Research has focused on evaluation and calibration of the Illinois Soil N Test for Iowa soils and climatic conditions. Initially, soil samples (0-6 or 0-12 inch depths from replicates of no-N control plots) from N rate trials at various sites in Iowa during 1999 and 2000 were analyzed by S. Khan at the University of Illinois to determine hydrolyzable amino sugar-N and/or the Illinois Soil N Test. These sites had multiple rates of fertilizer N applied to corn following either corn or soybean crops. Results of the soil analyses are compared to corn grain yield

response to applied N at each site (percentage yield increase above the yield from the control calculated at the 10:1 corn:nitrogen price ratio economic N rate). This way the sites can be classified as responsive or nonresponsive to applied N, and the test results can be compared to the critical levels indicated in the Illinois calibration for the hydrolyzable amino sugar-N or Illinois Soil N Test (Khan et al., 2001; Mulvaney et al., 2001). Illinois Soil N Test analyses continue on samples from these and other N rate studies conducted across Iowa.

As part of a larger statewide soil nitrogen and carbon management project, soil samples are collected from sites where multiple rates of N are applied to corn in producer fields. This project has been in place for the 2001 and 2002 seasons, and will continue in 2003. The field sites were chosen on criteria of diverse soils, range in yield potential, varying N management history, corn after soybean, no manure applied in the preceding fall or spring of that crop year, and minimum-till or no-till. Cooperators were asked to not apply N or manure to the area designated for the demonstration site. All other field activities are completed as normal by the cooperator. Fourteen sites were located for the project in 2001 and eleven in 2002. Six rates of N (0 to 200 lb N/acre in 40 lb increments) are applied shortly after planting (from planting to V2 growth stage) as surface applied ammonium nitrate. The N rates are replicated four times. No other N is applied except as noted for incidental N in starter or with phosphate fertilizer. Corn grain yield is measured by hand harvest and response to applied N determined (percentage yield increase above the yield from the control calculated at the 10:1 corn:nitrogen price ratio economic N rate). Each site is soil sampled for routine and soil N tests. Sampling for soil N tests includes fall, spring preplant, sidedress, and post-harvest. Soil is collected at 0-6 inch and 0-12 inch depths from either each replicate (fall or spring preplant sampling) or from each no-N control plot (sidedress or post-harvest sampling). Soil samples are frozen until air drying. The Illinois Soil N Test is being determined for each sampling and depth. Reported Illinois Soil N Test values for each site are the mean of the four field sample replicates and duplicate analyses of each sample.

### Preliminary Results

Table 1 gives the hydrolyzable amino sugar-N values for the soil samples analyzed by S. Khan at the University of Illinois. Using 250 mg kg<sup>-1</sup> as a critical level between responsive and nonresponsive sites (Mulvaney et al., 2001), the hydrolyzable amino sugar-N values correctly identified 6 of the 8 site-years as being N responsive. One site, Atlantic-LTN, was sampled each year and was nonresponsive to applied N each year. That site had a history of high swine manure application. Two sites had hydrolyzable amino sugar-N values >250, but the sites did respond to applied N. Based on the sites in 1999 and 2000, the hydrolyzable amino sugar-N analysis correctly identified the N responsiveness at 75% of the site-years. Shallower samples would be expected to have higher amino sugar-N levels. However, accounting for the sample depth differences would not change the indicated prediction of N response (Table 1).

Table 1 also shows the Illinois Soil N Test results for samples analyzed by S. Khan. The samples from 1999 were not run with the Illinois Soil N Test. These samples are being run in our lab, but results are not available at this time. For the five 2000 year sites with Illinois Soil N Test results, only one was identified correctly (using >235 mg kg<sup>-1</sup> as the critical level for predicted nonresponsive to applied N, Khan et al., 2001) as being nonresponsive (the Atlantic-LTN site). The other four sites had high Illinois Soil N Test values, but were responsive to applied N. As before, accounting for the differences in sample depth would not change the interpretation of N response. These preliminary results indicate that the measured hydrolyzable amino sugar-N level seems predictive of site N responsiveness, which is similar to the work reported by Mulvaney et al. (2001). However, the Illinois Soil N Test was not predictive of corn responsiveness to applied N. Analysis of the soil samples from the 1999 sites will help confirm this result. For those sites

that had both the hydrolyzable amino sugar-N and the Illinois Soil N Test analysis it appears that the values are not consistent between the two tests. One site was similar, but the other three sites had much higher Illinois Soil N Test values than hydrolyzable amino sugar-N (approximately 100 ppm higher).

Figures 1 and 2 show the relationship between the Illinois Soil N Test and corn yield response to N fertilizer at the 2001 and 2002 sites studied in the statewide soil nitrogen and carbon management project. Figure 1 has results for spring preplant soil samples collected from the 0-12 inch depth, and Figure 2 has results for spring preplant soil samples collected from the 0-6 inch depth. Based on a  $235 \text{ mg kg}^{-1}$  critical level for 0-12 inch samples, only one site would be predicted as responsive to applied N (Figure 1). That site was responsive but had only a small yield increase from N application, 5.7% increase above the control yield. All other sites had Illinois Soil N Test values above  $235 \text{ mg kg}^{-1}$  and would be predicted to be nonresponsive. However, of those 24 sites only 3 were nonresponsive to applied N. These results provide a correct prediction rate by the test of only 16%. Looking at the distribution of soil test values versus percent yield increase in Figures 1 and 2, one can easily see the lack of predictive relationship between the Illinois Soil N Test and corn yield response to applied N.

There would be great utility for a soil N test to have recommended sampling in the fall before planting corn. Figure 3 shows a good relationship between the Illinois Soil N Test values for soil samples collected in the fall and spring (values for the 0-6 and 0-12 inch depth samples included in Figure 3). This indicates the potential that soil sampling could occur either in the fall or spring. On-going work is also comparing test results for soil samples collected in-season.

One possible reason for the lack of predictive ability of the Illinois Soil N Test is the general relationship between the test values and soil organic matter (0-12 inch depth samples shown in Figure 4). A similar relationship exists for the 0-6 inch depth samples (data not shown). Across the sites in this study there was no correlation between soil organic matter and corn response to applied N (data not shown, but the relationship basically looks the same as that in Figures 1 and 2). In other words, soil organic matter was not predictive of N response. It is possible that the Illinois Soil N Test is reflecting general soil organic matter and not being specific to the amino sugar-N. This indication is seen in the results presented in Table 1 where the amino sugar-N determined by the soil hydrolysis procedure was much better related to N responsiveness.

#### Preliminary Conclusions

There appears to be a different pool(s) of soil organic-N measured by the hydrolyzable amino sugar-N procedure and the Illinois Soil N Test. This needs to be further studied by analyzing soil samples from these studies with both procedures. It is possible that the amino sugar-N fraction measured by soil hydrolysis will be a better predictor of N responsiveness than the Illinois Soil N Test, but that needs to be confirmed for these sites. This indicates that the underlying basis for the Illinois Soil N Test (hydrolyzable amino sugar-N) may be correct (can predict site N responsiveness), but the procedure developed for routine soil analysis (the Illinois Soil N Test) is not. At this point in time caution should be exercised with use of the Illinois Soil N Test for interpreting the need for N application in corn production (at least for Iowa soils). Further research is needed to clarify predictability of the test. Also, the soil samples from these studies need to be analyzed for exchangeable ammonium-N to see if that is contributing to inflated test results. Another year of field evaluation is planned, with additional soil analysis from other N rate studies conducted in Iowa.

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Table 1. Hydrolyzable amino sugar-N and Illinois Soil N Test for several N rate studies conducted in Iowa during 1999 and 2000.

| Site Name          | Previous Crop | Soil Sample |                   | Hydrolyzable                    | Illinois Soil       | Corn Yield             | N Fertilizer          |
|--------------------|---------------|-------------|-------------------|---------------------------------|---------------------|------------------------|-----------------------|
|                    |               | Time        | Depth             | Amino Sugar-N <sup>†</sup>      | N Test <sup>†</sup> | Without N <sup>‡</sup> | Response <sup>§</sup> |
|                    |               |             | inch              | ----- mg kg <sup>-1</sup> ----- |                     | bu acre <sup>-1</sup>  | %                     |
| Atlantic-LTN       | Corn          | Sp 1999     | 0-6               | 442                             | ---                 | 119                    | 0                     |
|                    |               |             | 6-12              | 350                             | ---                 |                        |                       |
|                    |               |             | 0-12 <sup>¶</sup> | 396                             | ---                 |                        |                       |
| Ames-LTN           | Corn          | Sp 1999     | 0-6               | 105                             | ---                 | 78                     | 170.7                 |
| Crawfordsville-LTN | Soybean       | Sp 1999     | 0-6               | 247                             | ---                 | 121                    | 32.3                  |
| McNay-LTN          | Soybean       | Sp 1999     | 0-6               | 215                             | ---                 | 80                     | 101.7                 |
| Ft. Dodge-SM       | Soybean       | Sp 2000     | 0-12              | 215                             | 319                 | 140                    | 20.0                  |
| Spencer-SMC1       | Soybean       | Sp 2000     | 0-12              | 378                             | 380                 | 142                    | 35.7                  |
| Sutherland-LTN     | Soybean       | Sp 2000     | 0-6               | 356                             | 441                 | 114                    | 18.6                  |
| Atlantic-LTN       | Soybean       | Sp 2000     | 0-12              | 305                             | 405                 | 158                    | 0                     |
| Ames-DIGM          | Corn          | Sp 2000     | 0-6               | ---                             | 316                 | 134                    | 14.1                  |
|                    |               |             | 6-12              | ---                             | 307                 |                        |                       |
|                    |               |             | 0-12 <sup>¶</sup> | ---                             | 312                 |                        |                       |

<sup>†</sup> Hydrolyzable amino sugar-N and Illinois Soil N Test determined by S. Khan, University of Illinois.

<sup>‡</sup> Corn yield with no N applied (control yield).

<sup>§</sup> Calculated as 100 x (optimal yield - control yield)/control yield.

<sup>¶</sup> Average of the 0-6 and 6-12 inch sample depths.

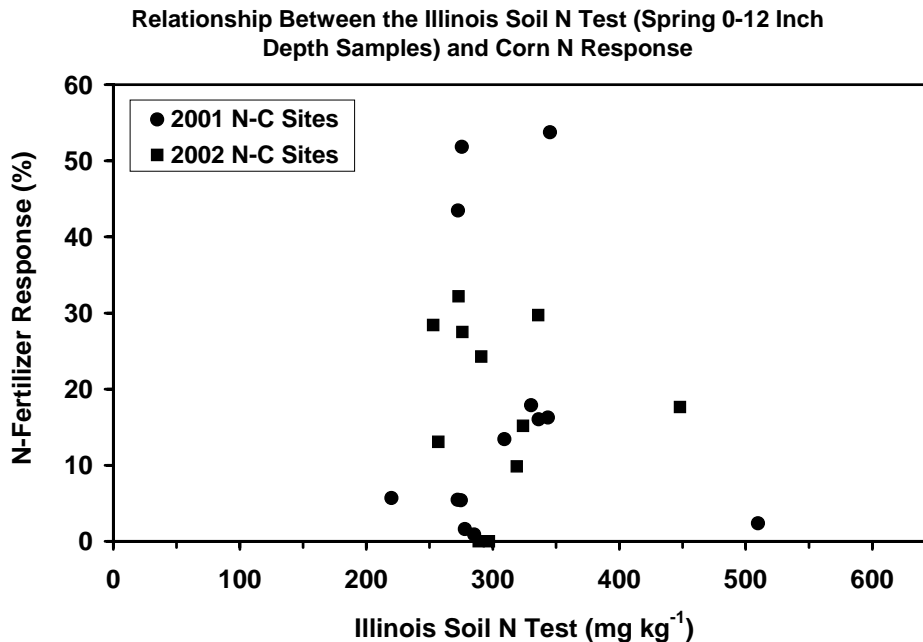


Figure 1. Relationship between the Illinois Soil N Test (0-12 inch soil sample depth) and corn yield response to applied N, preliminary results for 2001 and 2002. The N fertilizer response calculated as 100 x (optimal yield – control yield)/control yield).

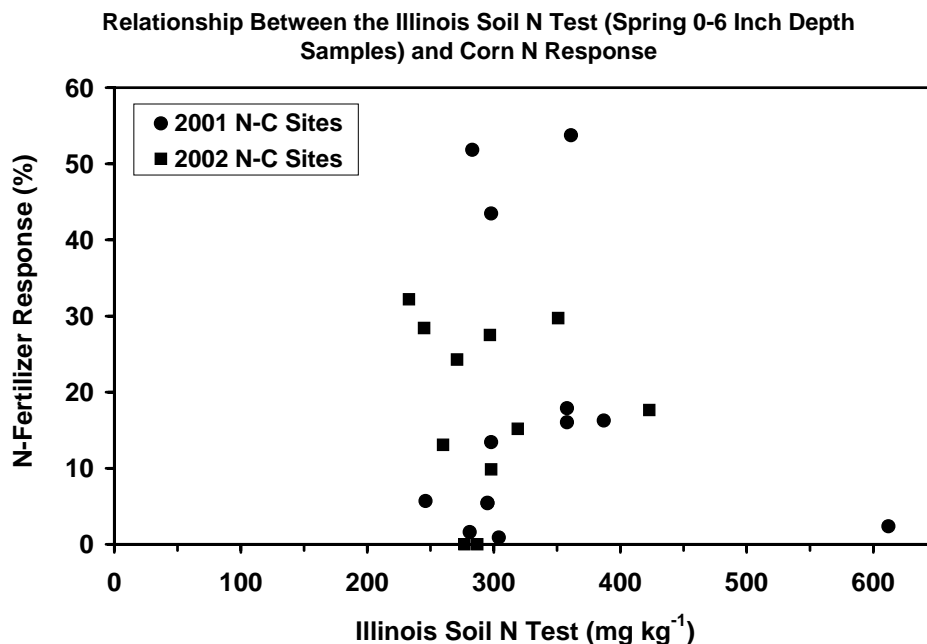


Figure 2. Relationship between the Illinois Soil N Test (0-6 inch soil sample depth) and corn yield response to applied N, preliminary results for 2001 and 2002. The N fertilizer response calculated as  $100 \times (\text{optimal yield} - \text{control yield}) / \text{control yield}$ .

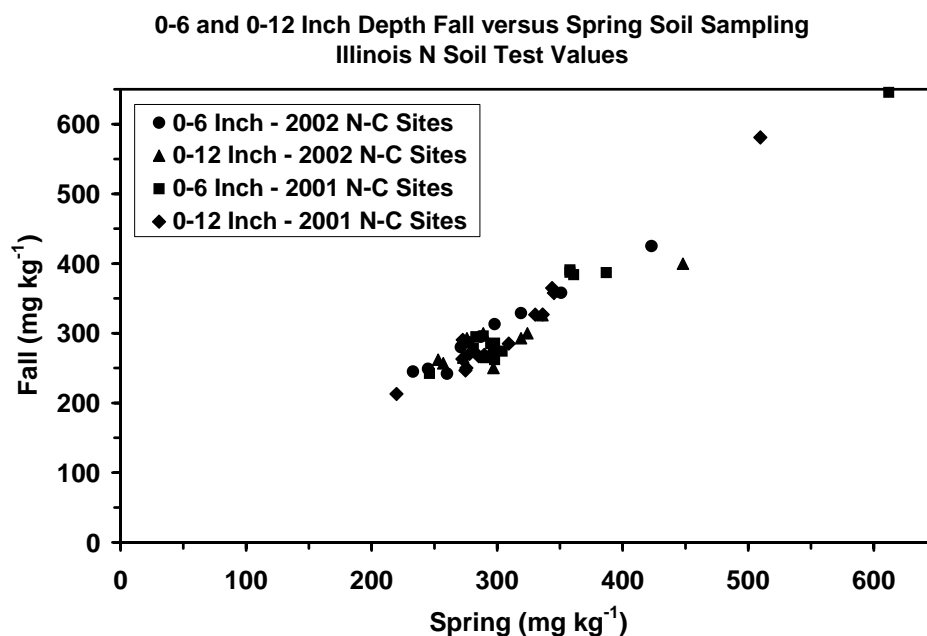


Figure 3. Relationship between the Illinois Soil N Test for 0-6 and 0-12 inch samples collected in the spring (preplant) and fall (either the fall before corn for 2002, or after corn harvest for 2001), preliminary results for 2001 and 2002.



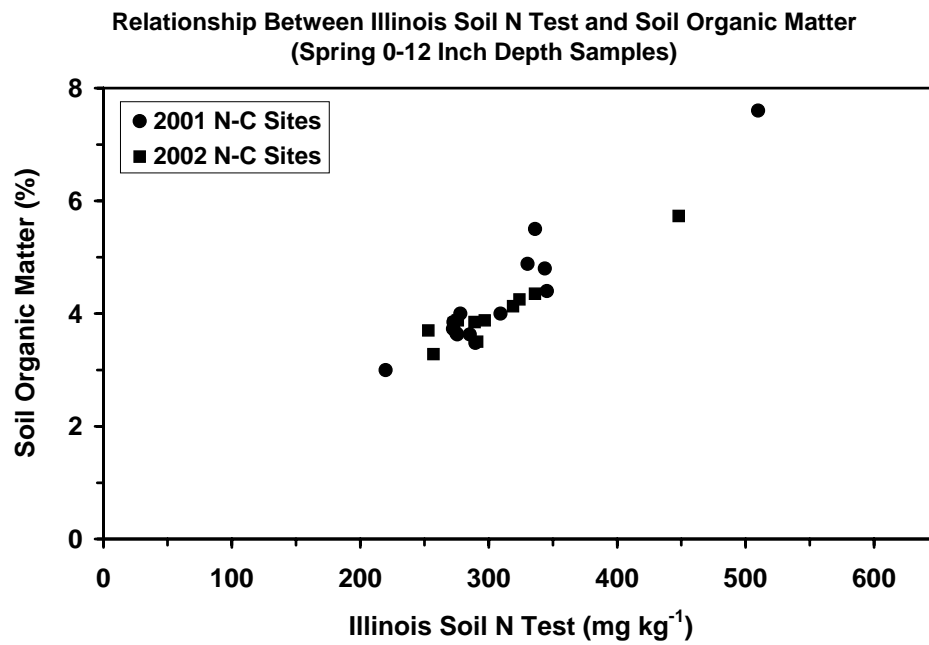


Figure 4. Relationship between the Illinois Soil N Test (spring 0-12 inch soil samples) and soil organic matter, preliminary results for 2001 and 2002.