EVALUATION OF THE AMINO SUGAR-N BASED SOIL TEST IN IOWA CORN PRODUCTION[1]

John E. Sawyer, Daniel W. Barker, John P. Lundvall, and Mahdi Al-Kaisi[2]
Iowa State University, Ames, Iowa

Abstract

An important improvement in estimating economic N applications for corn production would be to predict the soil N supply capacity each year. The objective of this research is to evaluate the adaptability and potential calibration of the amino sugar-N based Illinois N Soil Test in corn production fields across diverse Iowa soils. Nitrogen rate trials were conducted at multiple sites over several years, many on producer fields. Soil samples were collected at 0-6 and 0-12 inch depths in the fall and spring preplant where no N was applied. Samples were analyzed for hydrolyzable amino sugar-N (limited number of sites), the Illinois N Soil Test, soil organic matter, and total soil N. Corn response to fertilizer N was determined through change in grain yield. The hydrolyzable amino sugar-N values correctly identified six of eight site-years as being N responsive or non-responsive. Two sites had high test values, but were responsive to applied N. The Illinois N Soil Test values for each sample depth and sample timing were not related to corn yield response to applied N, and could not be calibrated on the soils studied. Also, the test did not differentiate between responsive and non-responsive sites. It is possible that the amino sugar-N fraction measured by soil hydrolysis is a better predictor of N responsiveness than the Illinois N Soil Test, but that needs to be confirmed by analysis of soil from additional sites. The Illinois N Soil Test values were similar for each sampling time. The Illinois N Soil Test did show a strong relationship with soil organic matter and total soil N. This indicates the test is reflecting multiple organic N pools or overall soil N rather than the specific amino sugar organic N fraction. Based on these results, the Illinois N Soil Test is not recommended for adjusting N applications to corn on Iowa soils.

Introduction

To improve estimates of economic N applications for corn production it would be helpful to predict the soil N supply capacity (ability to supply plant-available N) each year, especially for non-responsive fields. 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

[1] Presented at the 2003 North Central Extension-Industry Soil Fertility Conference, November 19-20, 2003, Des Moines, IA.
[2] Associate Professor and Soil Fertility Extension Specialist; Assistant Scientist; Extension Program Specialist, Assistant Professor and Soil Management Extension Specialist, Dept. of Agronomy, Iowa State Univ., 2104 Agronomy Hall, Ames, IA 50011-1010.

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, and 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 economic 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 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 non-responsive 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 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 non-responsive 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 N Soil 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, 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 non-response.

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 non-responsive – 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 mg kg^{-1}, and non-responsive at >250 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 N Soil Test). The Illinois N Soil 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 5 hours at 48-50ºC 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 N Soil 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 N Soil Test was <225 mg kg^{-1} and non-responsive if the test was >235 mg kg^{-1}. Test results are generally expected to be higher with 0-6 inch depth samples.

The Illinois N Soil Test Procedure

Details of the method and materials required to perform the test can be found in Khan et al. (2001). With a slight modification to jar positioning on the heating plate, this is also the procedure used in our laboratory. A 1.00 g sample of air-dried soil (ground to pass < 2mm) is weighed and placed to the bottom of a 1-pint Ball® Mason jar. A petri dish is suspended from a Mason jar lid, and 5 ml of 4% boric acid (H_3BO_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. The lid is immediately placed on the jar and sealed with a screw band. The sealed jar is transferred to a hot plate or griddle and heated for 5 hours at 48-50ºC (the temperature of the hot plate is pre-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 positioned so the lids are just touching. The jars are gently swirled and exchanged to a different position (where another jar had been located) on the hot plate once approximately half way through the 5-hour heating. Eight jars are placed in the middle portion of each hot plate, and when moved they occupy a front-back and an inside-outside position for the same length of time. At 5 hours the jars are removed from the hot plate. After an approximate 15 minute cooling period, the jars are opened, and the petri dish removed from the jar. 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 (H_2SO_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 N Soil 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, room temperature, 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.

Research in Iowa

Research has focused on evaluation and calibration of the Illinois N Soil 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 several 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 the Illinois N Soil Test. These sites had multiple rates of fertilizer N applied to corn following either corn or soybean. 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 allows the sites to be classified as responsive or non-responsive 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 N Soil Test (Khan et al., 2001; Mulvaney et al., 2001).

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, 2002, and 2003 years. 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, 11 in 2002, and 18 in 2003 (not all sites in 2003 have yield response information at the time of writing this report). 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). Yield relative to the maximal response to applied N is calculated for the no-N control. 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 if not immediately air dried. The Illinois N Soil Test is determined for each sampling and depth. Soil organic matter and total soil N are determined by dry combustion, with correction for inorganic carbon if necessary. Reported Illinois N Soil Test values for each site are the mean of the four field sample replicates and duplicate analyses of each sample.

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^{-1} as a critical level between responsive and non-responsive 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 both years and was non-responsive to applied N each year. That site had a history of high manure application. Two sites had hydrolyzable amino sugar-N values >250, but the sites responded 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 generally be expected to have higher amino sugar-N levels. However, accounting for sample depth differences would not change the indicated prediction of N response (Table 1).

Table 1 also shows the Illinois N Soil Test results for these sites. For the nine sites with Illinois N Soil Test results, one was identified correctly (using >235 mg kg^{-1} as the critical level for predicted non-responsive to applied N, Khan et al., 2001) as being non-responsive (Atlantic-LTN site) and one site as responsive (Ames-LTN). The other seven sites had high Illinois N Soil Test values, but were responsive to applied N. Accounting for differences in sample depth might change the interpretation of N response at the McNay and Armstrong-LTN sites. Four sites were sampled again in 2001. The Illinois soil N test values are similar to the prior sampling (Table 1).

These initial results indicate that the measured hydrolyzable amino sugar-N level seems reasonably predictive of site N responsiveness, which is similar to the work reported by Mulvaney et al. (2001). However, the Illinois N Soil Test was not predictive of corn responsiveness to applied N. For those sites that had both the hydrolyzable amino sugar-N and the Illinois N Soil Test analysis it appears that the values are not consistent between the two tests. One site was similar, but the other sites had higher Illinois N Soil Test values than hydrolyzable amino sugar-N (approximately 100 mg kg^{-1} higher).

Figures 1 and 2 show the relationship between the Illinois N Soil Test and corn yield response to N fertilizer at the 2001, 2002, and part of the 2003 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 mg kg^{-1} critical level for 0-12 inch samples, several sites predicted as responsive did have yield increase to applied N (Figure 1). However, sites with no N response also had values below 235 mg kg^{-1}. Also, many sites with test values above 235 mg kg^{-1} had large response to applied N. Looking at the scattered distribution of soil test values versus percent yield increase in Figures 1 and 2, one can see the lack of predictive relationship between the Illinois N Soil Test and corn yield response to applied N. The same situation occurs when comparing the soil N test values to relative yield of the no-N controls (Figure 3).

There would be utility for a soil N test to have recommended sampling in the fall before planting corn. Figure 4 shows a good relationship between the Illinois N Soil Test values for 0-6 and 0-12 inch soil samples collected in the fall and spring. This indicates the potential that soil sampling could occur either in the fall or spring.

One possible reason for the lack of predictive ability of the Illinois N Soil Test and poor correlation to relative yield is the positive relationship between the test values and soil organic matter and total soil N (Figures 5 and 6). 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 (Figure 7, which basically looks the same as the relationship in Figure 1). This is not surprising. It appears that the Illinois N Soil Test is reflecting overall soil N and not being specific to the amino sugar-N fraction or to a labile soil N pool that would reflect N responsiveness. This indication is also seen in the results presented in Table 1 where the amino sugar-N determined by the soil hydrolysis procedure was better related to N responsiveness.

Summary

There appears to be a different pool(s) of soil N measured by the hydrolyzable amino sugar-N procedure and the Illinois N Soil Test. This needs to be further studied by analyzing soil samples from more sites in these studies with both procedures. It is possible that the amino sugar-N fraction measured by soil hydrolysis is a better predictor of N responsiveness than the Illinois N Soil Test, but that needs to be confirmed for these sites. This indicates that the underlying basis for the Illinois N Soil Test (hydrolyzable amino sugar-N) may be correct (can predict site N responsiveness), but the procedure developed for routine soil analysis (the Illinois N Soil Test) is not. At this point in time caution should be exercised with use of the Illinois N Soil Test for interpreting the need for N application in corn production. Unless further analysis of soils and yield data from 2003 trials indicates otherwise, the Illinois N Soil Test is not recommended for prediction of corn N fertilization requirement or adjusting N applications to corn on Iowa soils.

Acknowledgement

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Table 1. Hydrolyzable amino sugar-N and Illinois N Soil Test for several N rate studies conducted in Iowa during 1999, 2000, and 2001.

Site Name	Previous Crop	Soil Sample Time	Soil Sample Depth	Hydrolyzable Amino Sugar-N[†]	Illinois N Soil Test	Corn Yield Without N[‡]	N Fertilizer Response[§]
			inch	- - - - - mg kg^{-1} - - - - -		bu acre^{-1}	%
Atlantic-LTN	Corn	Sp 1999	0-6	442	549	119	0
			6-12	350	261		
			0-12[¶]	396	405		
Ames-LTN	Corn	Sp 1999	0-6	105	233	78	171
Crawfordsville-LTN	Soybean	Sp 1999	0-6	247	326	121	32
McNay-LTN	Soybean	Sp 1999	0-6	215	259	80	102
Ft. Dodge-SM	Soybean	Sp 2000	0-12	215	319[†]	140	20
Spencer-SMC1	Soybean	Sp 2000	0-12	378	380[†]	142	36
Sutherland-LTN	Soybean	Sp 2000	0-6	356	441[†]	114	19
Atlantic-LTN	Soybean	Sp 2000	0-12	305	405[†]	158	0
Ames-DIGM	Corn	Sp 2000	0-6	---	316[†]	134	14
			6-12	---	307[†]		
			0-12[¶]	---	312[†]		
Armstrong-LTN	Soybean	Sp 2001	0-6	---	265	106	16
Ames-LTN	Soybean	Sp 2001	0-6	---	246	125	38
Crawfordsville-LTN	Soybean	Sp 2001	0-6	---	320	110	14
McNay-LTN	Soybean	Sp 2001	0-6	---	252	65	139
Sutherland-LTN	Soybean	Sp 2001	0-6	---	326	104	42

[†] Hydrolyzable amino sugar-N and Illinois N Soil Test determined by S. Khan, University of Illinois.

[‡] Corn yield with no N applied (control yield).

[§] Calculated as 100 x (10:1 corn:nitrogen price ratio economic yield - control yield)/control yield.

[¶] Average of the 0-6 and 6-12 inch sample depths.

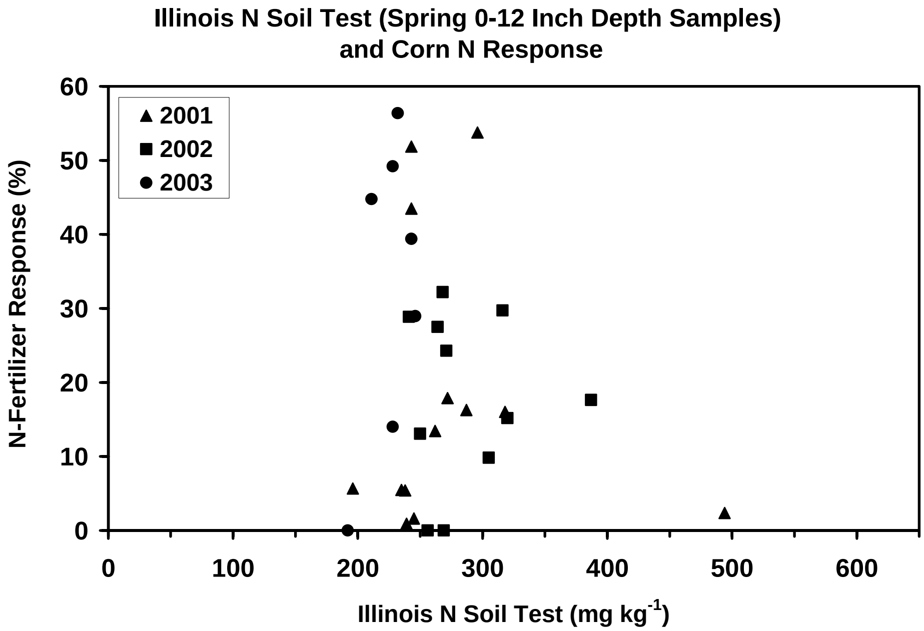

Figure 1. Relationship between the Illinois N Soil Test (spring 0-12 inch soil sample depth) and corn yield response to applied N, 2001 - 2003. The N fertilizer response calculated as 100 x (10:1 corn:nitrogen price ratio economic yield – control yield)/control yield.

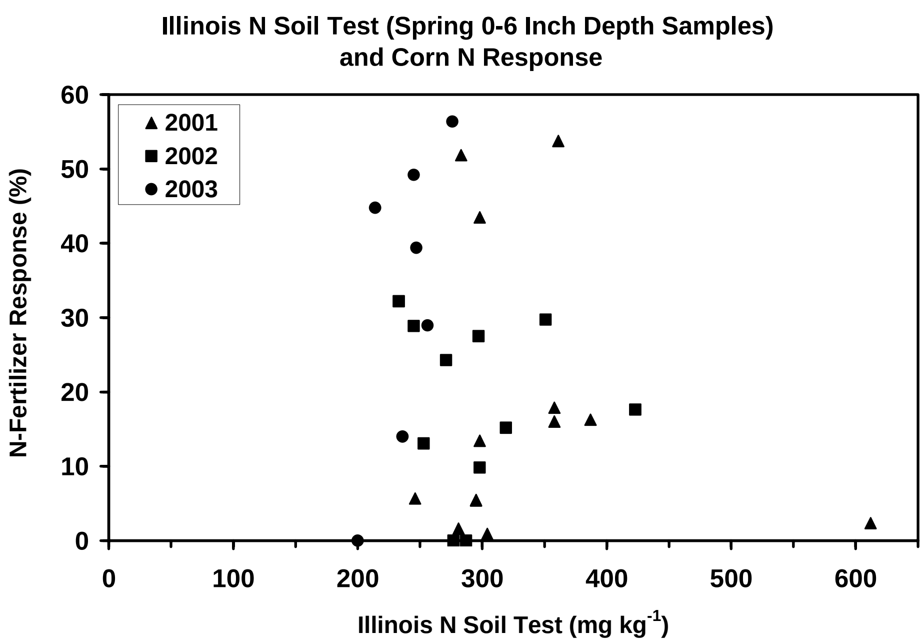

Figure 2. Relationship between the Illinois N Soil Test (spring 0-6 inch soil sample depth) and corn yield response to applied N, 2001 - 2003. The N fertilizer response calculated as 100 x (10:1 corn:nitrogen price ratio economic yield – control yield)/control yield.

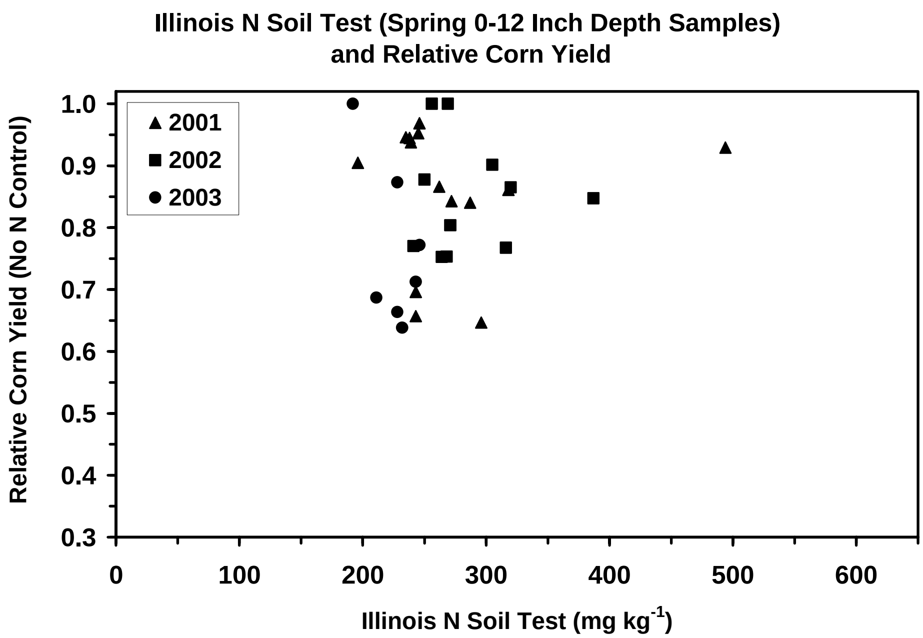

Figure 3. Relationship between the Illinois N Soil Test (spring 0-12 inch soil sample depth) and relative corn yield of the no-N control, 2001 - 2003. Relative yield calculated as control yield/yield at maximum response.

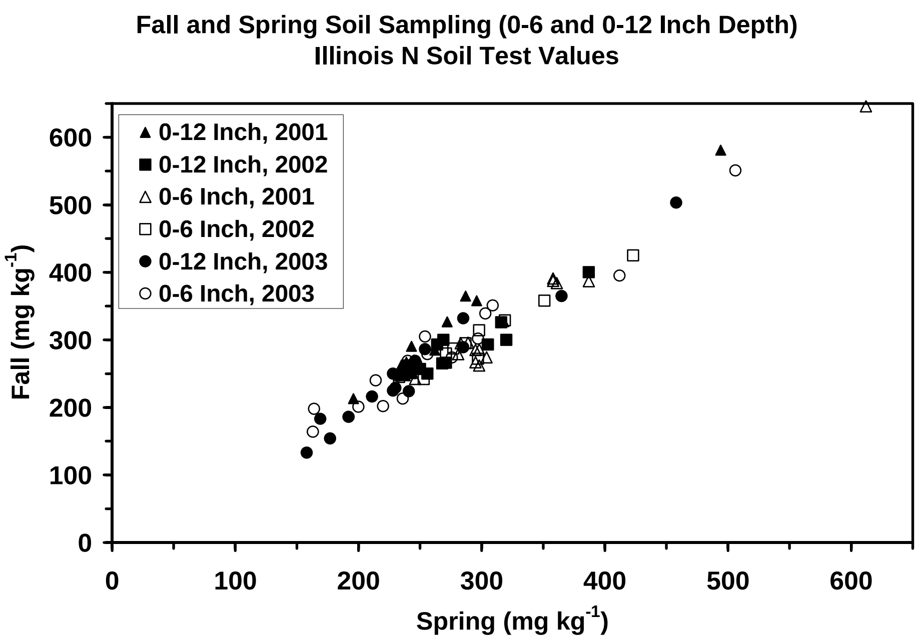

Figure 4. Relationship between the Illinois N Soil Test for 0-6 and 0-12 inch samples collected in the spring (preplant) and fall (either the fall before corn for 2002 and 2003, or after corn harvest for 2001), 2001 - 2003.

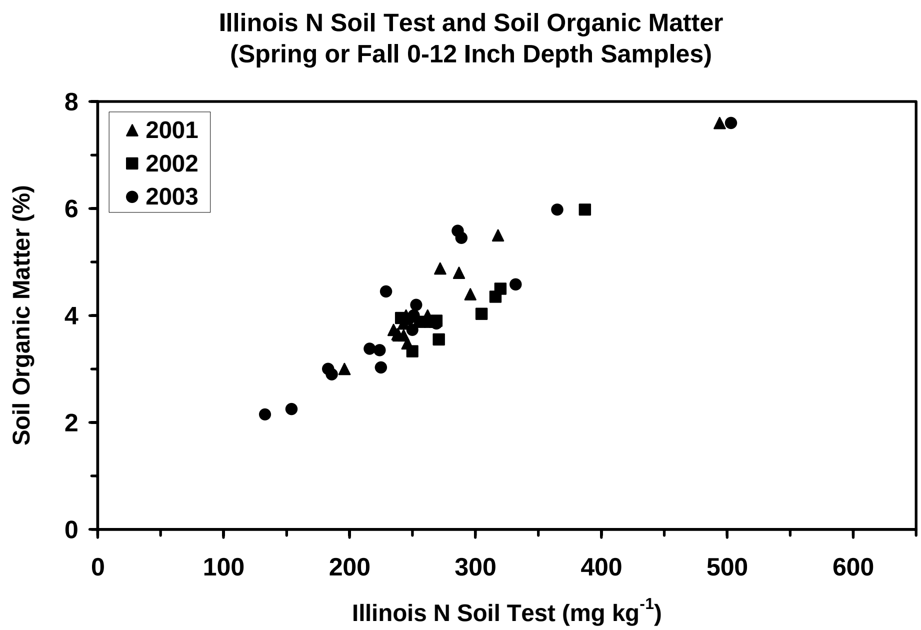

Figure 5. Relationship between the Illinois N Soil Test (spring or fall 0-12 inch soil samples) and soil organic matter, 2001 - 2003.

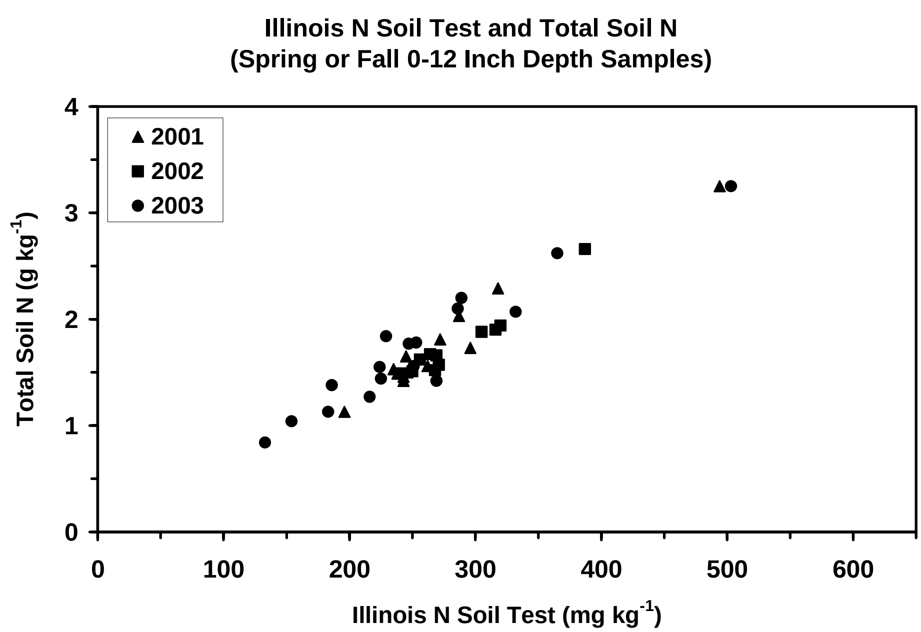

Figure 6. Relationship between the Illinois N Soil Test (spring or fall 0-12 inch soil samples) and total soil N, 2001 - 2003.

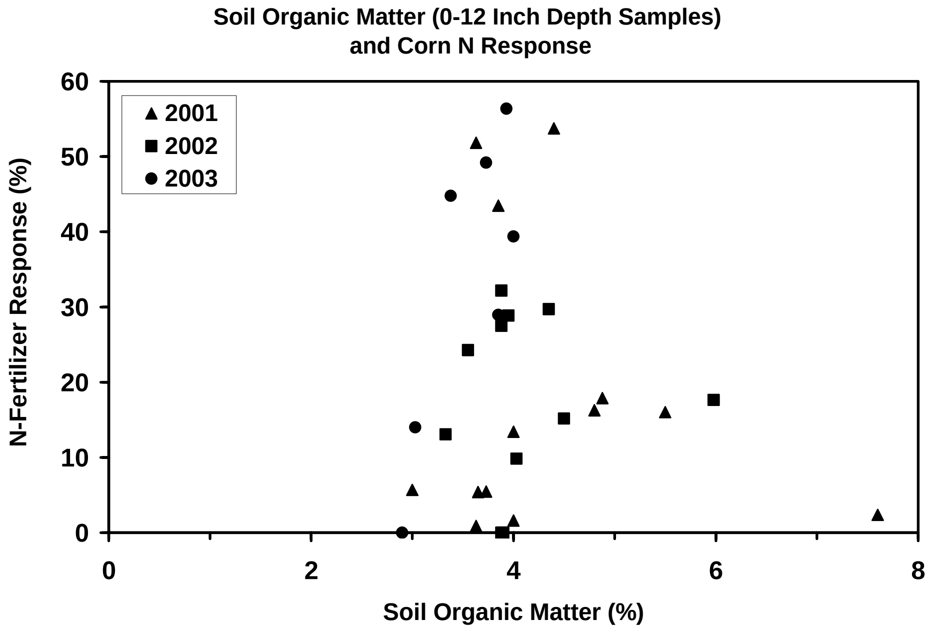

Figure 7. Relationship between soil organic matter (0-12 inch soil sample depth) and corn yield response to applied N, 2001 - 2003. The N fertilizer response calculated as 100 x (10:1 corn:nitrogen price ratio economic yield – control yield)/control yield.