Chapter 4

Recommended Soil Nitrate Tests

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A number of soil testing laboratories in the Northeast Region offer testing for soil nitrate to provide an estimation of the nitrogen supplying capacity of the soil to crop plants. The analysis most commonly carried out is the Presidedress Soil Nitrate Test (PSNT) which is an in-situ incubation method used to determine the probability of crop response to nitrogen fertilization (Gelderman, R. H. and D. Beegle. 1998). Typical spring rainfall amounts in the Northeast preclude the use of a preplant soil nitrogen test, which is recommended in the Great Plains and in some of the more humid areas of the Midwest (Bundy et al., 1994; Schmitt and Randall, 1994).

The PSNT was originally developed in Vermont (Magdoff et al., 1984) for field corn. Throughout the following decade, the ability of the PSNT to predict nitrogen fertilizer response and its usefulness in developing nitrogen fertilizer recommendations were the subjects of numerous research studies in the Northeast (Magdoff et al., 1990; Fox, et al. 1989; Meisinger et al., 1992; Klausner et al., 1993; Sims et al., 1995). Field corn continued to be the crop of interest in these initial studies.

More recent research activities have successfully developed and interpreted PSNT calibration data for vegetables including pumpkins (Morris et al., 2000), sweet corn (Heckman et al., 1995), peppers (Howell, 1999), fall cabbage (Heckman et al., 2002) and other cool season crops such as broccoli, cauliflower, lettuce and celery (Hartz and Breschini, 2000; Hartz and Bendixen, 1998). The test has been found to be particularly useful in determining whether additional nitrogen should be supplied to crops grown on sites where nitrogen is derived from soil amendments like composts, manures, natural organic fertilizers or legume rotations (Heckman, 2002; Grubinger, 2005).

Most research was completed on soils where no or only a small amount of nitrogen fertilizer (< 20 lbs N acre^{-1}) was applied before sample collection for the PSNT. Application of fertilizer could make it difficult to interpret the test because it would be impossible to distinguish the nitrate from the fertilizer and the nitrate from mineralization of soil organic matter. The fertilizer application would confound the in-situ incubation as proposed by Magdoff et al. (1990).

Some studies have been completed, however, with numerous rates of nitrogen fertilizer applied before planting to establish different concentrations of nitrate in the soil (Blackmer et al., 1989; Binford et al., 1992; Morris et al., 2000). Surprisingly, the critical concentrations of nitrate-N for the experiments where nitrogen fertilizer was applied before planting are similar to the 20 to 25 mg N kg^{-1} range calculated for experiments where no fertilizer was applied before planting.

There has been sufficient research to recommend the PSNT be included as part of a Best Management Practice (BMP) for crops where sufficient calibration data are available. Careful interpretation of the results are required, however, because rainfall before and after the collection of soil samples for the PSNT can substantially change the soil nitrate concentration and the amount of nitrogen fertilizer needed for optimum yield. Widespread use of the PSNT would improve the efficiency of nitrogen management for optimum plant production and minimal groundwater contamination (Guillard et al., 1999).

The PSNT requires stricter sampling and handling protocols than typical soil macro-nutrient tests. For most crops, soil samples for the PSNT are collected immediately before the plant begins rapid nitrogen uptake. This occurs when corn plants are 8 to 12 inches (20 to 30 cm) tall. A listing of the appropriate plant phenological times for collecting soil samples for various vegetable crops is found in Heckman, 2002.

Soil samples for the PSNT are collected from the top one foot (30 cm) of soil. This increased depth of sampling, relative to standard soil fertility tests, is because of anticipated differences in vertical nitrate-N distribution attributed to variation in spring rainfall (Binford et al., 1992; Griffin et al., 1995). Soil microbial activity can change inorganic soil nitrate concentrations through mineralization of organic N or denitrification of nitrate. To minimize soil microbial activity, samples are placed in cloth bags (distributed for free by many labs), which facilitates drying of the samples during shipment. Samples should be brought or sent to the laboratory within 3 days of sampling. Shipment of samples in cloth bags minimizes changes in nitrate in the samples for up to 3 days (Morris, 1998). If samples are to be stored for a day or two, the samples should be refrigerated. Samples can also be dried before shipment to the laboratory. Drying is best accomplished by spreading thinly (< 1-cm thick) and air drying or by oven drying at 60^{o}C (Griffin et al., 1995). Field-moist samples received by a laboratory should be immediately spread to air dry or placed in an oven to dry.

According to Griffin et al. (1995), water or dilute salt solutions can be used to extract nitrate from most soils because essentially all the nitrate in soils with low anion exchange capacities is water soluble. The main disadvantage of water is its low ionic strength which can cause dispersion and result in cloudy filtrates. Extractants containing chloride cause problems if nitrate-N is measured by ion chromatography or ion selective electrode because chloride can interfere with analysis of nitrate-N by these methods. If exchangeable and water-soluble ammonium-N are to be measured, or if the soils being analyzed have appreciable anion exchange capacity, 2 M KCl is an ideal extractant (unless nitrate-N is measured by ion chromatography or ion selective electrode).

Recommended Extractants and Extracting Procedures for Soil NO_3^--N

Equipment:

1. Instrumentation for analysis of NO_3^--N (e.g. An automated ion analyzer or continuous flow system, ion chromatograph, ion selective electrode, steam distillation glassware).
2. Reciprocating shaker, capable of 200 opm (oscillations per minute).
3. Electronic balance.
4. Erlenmeyer flasks (125 mL).
5. Filter funnels.

Extractants – *Select based on analytical technique to be used*:

0.01 *M* $CaSO_4$.

This extractant provides clear filtrates and the sulfate in the extract does not interfere with analysis for NO_3^- -N by most methods, including ion chromatography. Since this is a relatively dilute solution, salt encrustations in the lab (including occasional freezing of syringes in automatic pipetting machines) associated with "high salt" extractants are avoided. To prepare, dissolve 1.72 g of $CaSO_4 \cdot 2H_2O$ in 1 L of deionized water.

OR

2*M* KCl.

This is the extractant recommended by Keeney and Nelson (1982) to extract exchangeable NH_4^+-N. When NO_3^- -N and NH_4^+ -N are both to be measured in soil samples, 2 *M* KCl is the extractant of choice. Because of its high Cl^- content, 2 *M* KCl also effectively extracts exchangeable NO_3^- -N from soils. To prepare, dissolve 150 g of KCl in 1L deionized water.

OR

0.01*M* $CaCl_2$.

This extractant provides clear extracts. It is recommended for ion chromatography extracts when sulfate interference is of concern. According to Griffin (1983), $CaCl_2$ can be used for the Cd reduction procedure. To prepare, dissolve 1.47 g of $CaCl_2$ in 1 L deionized water.

OR

0.04 *M* $(NH_4)_2SO_4$.

This is the preferred extractant if the nitrate selective electrode is used. The solution contains H_3BO_3 as a preservative. Directions for preparation are listed under the Nitrate Electrode Method heading of this chapter.

Extraction Procedure:

1. The soil:solution ratio for NO_3^- -N extraction depends on the analytical method used. For instruments with low detection limits (e.g. autoanalyzer or ion chromatograph) the recommended ratio is 1:10 (5 g soil:50 mL extractant). For ion selective electrodes the recommended ratio is 1:2.5 (20 g soil:50 mL extractant).

2. Weigh the appropriate amount of air-dried, ground and/or sieved (2mm) soil into a 125 mL Erlenmeyer flask.

3. Add 50 mL of the desired extractant (0.01 *M* $CaSO_4$, 2 *M* KCl or 0.04 *M* $(NH_4)_2SO_4$ or 0.01 *M* $CaCl_2$).

4. Shake for 15 minutes on a reciprocating shaker at 200 oscillations per minute.

5. Filter the soil suspension using any medium grade filter paper (e.g. Whatman No. 2) that will provide a clear filtrate without contributing measurable amounts of NO_3^--N to the filtrate.

Recommended Analytical Procedures for Measuring Soil NO_3^--N

Numerous methods of measuring NO_3^- -N in soil extracts are described by Keeney and Nelson (1982), Those that have been used in the northeast region include the nitrate specific electrode, ion chromatography, steam distillation, and colorimetric procedures in which NO_3^- -N is measured either directly or after reduction to nitrite (NO_2^- -N). The most commonly used procedures in university soil testing labs in the Northeast are colorimetric determination after cadmium (Cd) reduction and the nitrate electrode. In addition, some labs are using or have had experience with test kits (e.g. Nitracheck meter, Cardy meter, and Hach kit) used to measure soil NO_3^- in the field (for example, see Jemison and Fox, 1988).

The nitrate electrode method, while attractive because of speed, apparent simplicity, and relatively low cost of required equipment, has some shortcomings. As stated by Keeney and Nelson (1982), "nitrate electrodes are subject to numerous interferences and to subtle variations in sample handling, electrode calibration, reference electrodes, electrode preparation, and perhaps even differences among NO_3^--N electrode supplied by the same manufacturer." Some of the more commonly used extractants for NO_3^--N, including 2 *M* KCl, cannot be used due to Cl^- interference. Acetate also interferes. Finally, because of the low sensitivity of the electrode, relatively small soil:extractant ratios (e.g. 1:2.5) are required to measure NO_3^--N in many soils (Gelderman and Fixen, 1988). Despite these concerns, some laboratories in the Northeast have developed successful procedures that use the nitrate electrode for the analysis of routine soil samples (Table 4-1). Wilhelm et al. (2000) reported satisfactory nitrate electrode use with the end-of season cornstalk tissue test.

Most of the analytical procedures described above, when properly conducted, will accurately measure NO_3^--N in soil extracts. Automated procedures, such as the cadmium reduction method and ion chromatography, offer the advantages of speed as well as accuracy. Some considerations with regard to these methods are presented below. A procedure for the nitrate electrode is also described, and this may be more suitable for laboratories analyzing only a small number of samples per year due to the instrumentation costs and greater setup time required with the automated instruments.

Table 4-1. Comparison of three methods of determining nitrate in soil extracts. Results of 1990 NEC-67 sample exchange.

	Analytical Method				
	Ion Chromotography		**Cd Reduction**		**Ion Electrode**
Soil	**Lab 1**	**Lab 2**	**Lab 1**	**Lab 2**	**Lab 1**
	---------------------------------- mg NO_3-N kg soil^{-1} ------------------------------				
1	24	24	28	28	28
2	68	65	74	74	65
3	2.5	2.0	2.1	< 4	3.5
4	1.3	1.1	1.0	< 4	2.3
5	14	15	15	15	15
6	32	33	36	36	35
7	85	82	83	83	88

The Cadmium Reduction Method

The Cd reduction method involves reducing NO_3^--N to NO_2^--N in a “cadmium column” containing copperized Cd. The NO_2^--N is then measured colorimetrically following reaction with a diazotizing reagent (sulfanilamide) and a coupling reagent [N-(1-naphthyl) – ethylenediamine dihychloride] following a modified Griess-Illosvay method. A pinkish-purple color develops that is then measured between the wavelengths of 510 and 550 nm; maximum sensitivity is approximately 540 nm (Keeney and Nelson, 1982).

The Cd reduction method is very sensitive and free from interferences from organic matter and soil cations (Keeney and Nelson, 1982). Dorich and Nelson (1984) reported the minimum reproducible concentration of NO_3^--N that could be detected in soil extracts by a manual Cd reduction procedure was 0.014 mg NO_3^--N/L. The maximum concentration that could be measured without dilution was 20 mg NO_3^--N/L. The authors obtained a coefficient of variation for the manual method that ranged from 2.1 to 3.4 %. They also reported that 36 sample extracts could be analyzed per hour if four reduction columns were used. Manual Cd reduction methods are described by Huffman and Barbarick (1981), Keeney and Nelson (1982) and the U.S. E.P.A. (1974).

Automated procedures for measuring NO_3^--N by Cd reduction have been reported by Henricksen and Selmer-Olsen (1970), Skjemstad and Reeve (1978) and the U.S. E.P.A. (1974). More recently developed procedures are described by the manufacturers of the various instruments that perform automated analyses. The automated procedures offer the advantage of speed (from 20 to 120 sample extracts per hour, depending on manufacturer and vintage of the equipment), good precision and uniformity of flow rate through the reduction column from sample to sample. New automated equipment, however, is expensive, and good used or rebuilt equipment is often difficult to find.

The working concentration range for some automated Cd reduction systems is only 0 to 2 mg NO_3^--N per L of solution analyzed. For those systems, analysis of soil extracts with NO_3^--N concentrations above 2 mg/L requires either diluting the extracts manually, adjusting the size of the sample and diluent lines on the manifold, or adding a pre-dilution loop to the manifold. Many newer automated systems have increased the working concentration range (to 0 to 20 mg/L, for example), thereby avoiding the problem. Experience in Connecticut with the PSNT has shown that the NO_3^--N concentration range for most soil extracts is 0.5 to 6 mg NO_3^- -N/L for a 1:10 (w:v) soil:extractant ratio.

Equipment/Procedure/Reagents:

Standard solutions used in CT: To prepare 250 mg NO_3^- -N/liter matrix matched stock solution, add 1.8045 g oven-dry potassium nitrate (KNO_3) to l L volumetric and fill to volume with 0.01*M* $CaCL_2$ extracting solution. Keep stock solution and working standards refrigerated.

Working Standard	mL of 250 mg NO_3^- -N/liter to add
0.5	0.5
1.0	1.0
2.0	2.0
4.0	4.0
6.0	6.0
10.0	10.0

Make standards in 250 ml volumetric flasks and fill to volume with 0.01 *M* $CaCl_2$.

Nitrate Electrode Method

Soils are extracted with a 0.04*M* $(NH_4)_2SO_4$ solution to maintain constant ionic strength and the extracts are measured for nitrate-N with a specific ion electrode. When placed in a nitrate-containing solution the electrode develops a potential, the magnitude of which is dependent on the difference of nitrate in the sample and reference solution. The nitrate electrode measures the level of free nitrate in solution.

Equipment:

1. Nitrate selective ion electrode and meter.
2. Magnetic stirrer and stir bars.

Reagents:

1. **Ionic strength adjustor (ISA):** Used to maintain constant ionic strength. Prepare a 2*M* $(NH_4)_2SO_4$ solution by dissolving 26.4 g of reagent grade $(NH_4)_2SO_4$ in 100 mL of distilled water.

2. **Preservative solution:** Prepare a 1*M* H_3BO_3 solution by dissolving 6.2 g of H_3BO_3 in 100 mL of boiling distilled water. Cool, transfer to a 100 mL volumetric flask and make to volume with distilled water.

3. **Extracting solution (0.04*M* $(NH_4)_2SO_4$):** Add 20 mL of ISA and 10 mL of preservative solution to a 1 L volumetric flask. Make to volume with distilled water.

4. **Reference Electrode Outer Chamber Filling Solution:** Add 2 mL of ISA to 100 mL of distilled water. Use to fill outer chamber of reference electrode.

5. **Standard Nitrogen Solutions:** Prepare a 1000 mg NO_3^-N/L standard solution by dissolving 7.22 g of KNO_3 in a 1 L volumetric flask with distilled water and diluting to volume. Prepare 250 mL of a 100 mg NO_3^-N/L stock solution by diluting this solution 10:1.

6. **Working NO_3^-N standards:** Prepare by diluting the following mls of the indicated stock solution to volume in 500 mL volumetric flasks with distilled water:

Working Standard (mg NO_3^-N /L)	**NO_3^-N Stock Solution (mL)**	**ISA (mL)**	**Preservative (mL)**
4	20 mL of 100 mg NO_3^-N /L	10	5
10	50 mL of 100 mg NO_3^-N /L	10	5
40	20 mL of **1000 mg** NO_3^-N /L	10	5

Procedure:

1. Obtain soil extract for NO_3^--N analysis as described previously.

2. Set specific ion meter to calibration mode and calibrate using the 4 mg NO_3^-N /L and the 40 mg NO_3^-N /L standards. Stir all solutions with a magnetic stirrer at moderate speed during calibration and analysis. After calibration, check the concentration of the 10 mg NO_3^-N /L standard.

3. Measure the NO_3^--N concentration in the soil extracts. Multiply the result by 2.5, or by the appropriate extract:soil ratio to determine the NO_3^--N concentration in mg/kg soil.

Interpretation of Results for PSNT Use

The PSNT is primarily used by growers to discern whether a crop will respond to sidedress fertilizer N. It is used by agronomists to improve N fertilizer recommendations and can also provide information about residual and post-harvest soil nitrate. Improved, site-specific N fertilizer recommendations will maximize N fertilizer efficiency while concurrently minimizing groundwater contamination by nitrate (Hartz and Breschini, 2000). Timely N fertilizer recommendations using in-season tests like the PSNT require that the tests be performed at the recommended critical crop growth stage and the results should be rapidly available (Heckman, 2002), because growers often obtain the recommendations by cell phone while waiting in the field to be fertilized

For the crops that have been studied to date, including silage corn, sweet corn, pumpkins, cole crops, peppers, lettuce and celery, a soil NO_3^--N concentration in the range of 25 to 30 mg/kg indicates sufficiency but specific fertilizer recommendations may be modified by local Cooperative Extension specialists (Heckman, 2002).

Use of the PSNT to estimate sidedress nitrogen fertilizer requirements is more appropriate under certain agronomic or site situations than others. Factors to consider when identifying sites where this test would be valuable include (Heckman, 2002):

1. Coarse soils with low organic matter content often mineralize only small amounts of nitrate, and in humid regions like the Northeast US nitrate that is mineralized in coarse soils is easily leached. Soil nitrate concentrations are typically low in these soils, and little useful information is obtained by performing the PSNT on these soils.

2. Manure or compost amended soils increase the mineralization potential of soils and make these soils good candidates for the PSNT. How much the soil mineralization potential has been increased is difficult to predict. The PSNT reduces the uncertainty.

3. Application of preplant broadcast N is susceptible to leaching in humid regions. The PSNT can be used to check if there is sufficient nitrate in the soil is heavy rainfall has occurred after application of preplant fertilizer.

4. Soils double cropped with a late summer crop like cabbage after sweet corn will sometimes have sufficient carryover of nitrate in the soil to reduce the amount of nitrogen fertilizer needed for the second crop. The PSNT can estimate the carryover and provide information about the amount of nitrate available from the incorporation of residue from the first crop.

5. Legume crops and cover crops can supply large amounts of nitrate to soils. The timing and amount of nitrate available from the decomposition of the legume residues is difficult to predict. The PSNT can reduce the uncertainty about nitrate availability from legumes.

6. Organic nutrient management systems rely almost entirely on nitrogen mineralization from organic amendments for their nitrogen supply. The PSNT can be used to estimate if sidedress nitrogen is needed in these systems or if more or less organic amendments are needed in the future. The options for sidedress application of nitrogen in these systems is limited because there are few materials with a high percentage of soluble nitrogen, but some materials are available. The most important use of the PSNT in organic systems is to ensure sufficient but not excess nitrate availability from organic amendments.

The PSNT is a diagnostic tool that can reduce uncertainty in N management, but it cannot predict the magnitude of yield responses in individual fields. The test was not developed to predict future weather events after the samples are collected and the fertilizer is applied. Drought or excessive rainfall later in the season can reduce yields and yield responses to N, and the test results cannot be expected to provide accurate recommendations when unusual weather events occur after sampling. Understanding what the PSNT can do and cannot do is crucial when using the test to guide sidedress N fertilizer recommendations.

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