Field Measurement of Soil Nitrate Concentrations

Rob Wetselaar,* Geoffrey D. Smith,* and John F. Angus

*Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra, ACT 0200, Australia
bDivision of Plant Industry, CSIRO, Canberra, ACT 2601, Australia

ABSTRACT

Information on the nitrate concentration of soil at the time of sowing crops is useful in estimating the optimal amount of fertilizer nitrogen (N). A field measurement of soil nitrate overcomes the delay and cost of laboratory analysis. The accuracy of Merckoquant test strips used with a hand-held Nitracheck reflectometer was tested with a wide range of soil samples. We have found that the system gave accurate results provided two precautions were taken. First, samples should be compared with standard solutions analyzed at the same temperature, because the method was found to be temperature dependent. Second, the extractant should be 0.5M K_2SO_4 or water, since the usual soil extractant, KCl, interferes with the analysis. The use of water for extraction reduces the cost of analysis and makes it feasible to analyze kilogram amounts of soil, thereby reducing the error of subsampling. We report a procedure which minimizes the time of extraction and hence the chance of denitrification, by filtering the suspension of soil in water through a 0.8-mm filter attached to a syringe.
INTRODUCTION

The optimal amount of nitrogen fertilizer for crops can be estimated using data on the amount of mineral N present in the soil at about the time of sowing and the amount likely to be mineralized during crop growth (Myers, 1984). It is important to make an accurate measurement of the former because it varies much more from season to season than the latter (unpublished results). It is also important to minimize the delay between measurement and fertilizer application to avoid errors due to nitrate losses or additional mineralization after measurement.

The two forms of mineral N are nitrate and ammonium. In most cases the ammonium concentration in the soil is so low that it can be ignored. Laboratory methods for measuring soil nitrate involve the cost of transporting samples and the delay in obtaining the results, so a field test has advantages. Consequently, in this study we have concentrated on developing a field method for extraction and measurement of soil nitrate with comparable accuracy to laboratory methods.

The two field methods tested by Sims et al. (1995) were the Cardy meter, a specific nitrate-electrode method, and a reflectometer method using a Nitrachek reflectometer in conjunction with Merckoquant nitrate test strips. Both methods employ battery-operated, hand-held instruments which report the nitrate concentration on digital displays; both were originally intended for field measurement of nitrate concentration of fresh plant material, but have been adapted to soil nitrate measurement. Both require only a few drops of liquid for analysis. In the case of the Merckoquant/Nitrachek system, colloid or coloring must be removed because of the interference with the reflected light from the test strip. The problems of colloid and coloring do not apply to the Cardy meter. However, Sims et al. (1995) concluded that measurements of soil nitrate using the Merckoquant/Nitrachek method correlated better with laboratory measurements than with the Cardy meter.

Other field methods (Jemison and Fox, 1987; Oehmichen, 1987; Roth et al., 1991), using the Nitrachek reflectometer are available, but all of these have drawbacks such as restriction of the soil subsample size due to the use of soil extracting solutions with flocculating properties, a risk of denitrification due to a 4- to 6-hour extraction procedure with water as the extractant, and not taking into account the effect of temperature on the method.

MATERIALS AND METHODS

The Merckoquant 10020 test strips (E. Merck, 64271, Darmstadt, Germany) consist of white plastic strips with dimensions 74x6 mm, at one end of which are two 6x6 mm white paper pads containing reagents that develop a red-violet color. The distal pad is sensitive to nitrate plus nitrite and the proximal pad is sensitive to nitrite alone. The concentrations can be roughly estimated by visually comparing the pads with seven color standards printed on the container, after color
development on the test strip for 60 seconds. The Nitracheck™ reflectometer (model 404, Quomed Ltd., England) enables the nitrate concentration to be read more accurately than by visual comparison with the standards, but is not designed to detect the nitrite on the second pad on the strip. The color development is sensitive to nitrate concentrations in the range from approximately 5 to 300 mg NO$_3^-$ L$^{-1}$. When the volume of the filtered extract is limited, a small tube with an I.D. of 8 mm can be used, requiring only about six drops of the filtrate for the distal pad of the strip to be moistened by the filtrate.

The measurement on the strip involves reduction of nitrate to nitrite by an unspecified reducing agent. The nitrite formed produces nitrous acid in the presence of a buffer, and the acid diazotizes an aromatic amine. Coupling with N-(1-naphthyl)ethylenediamine produces a red-violet azo dye. This method is similar in principal to a method of laboratory nitrite analysis (Bremner, 1965). The instruction leaflet for the Merckoquant strips mentions precautions to be taken with measurements at pH values between 1 and 12, but does not give precautions concerning the effect of temperature on the measurements. With relation to interference of other ions, the manual indicates that as little as 0.5 mg L$^{-1}$ nitrite produces an apparent nitrate reading. Its interference can be eliminated by mixing 2 mL of the sample with one drop of 10% aqueous amidosulphonic acid solution. After shaking well, the mixture can be tested for nitrate after two minutes. Other interferences are described in the manual, but virtually none of these is likely to occur in samples taken from natural or agricultural environments.

Several filtration methods were tested for preparing clear soil nitrate extracts with water as the extractant. We found the most convenient to be the use of a 25-mL plastic syringe of which the outlet is connected to the inlet of a disposable 26-mm diameter, 0.8-m cellulose acetate sterile filter such as those supplied by Sartorius AG, Germany, or other suitable company.

RESULTS

Comparison of Reflectometer Method for Water-Extracted Soil with Standard Methods

The accuracy of field determination of the reflectometer method using a water extract was tested by comparing it with an autoanalyzer method (Markus et al., 1985) and a distillation method (Bremner and Keeney, 1966; Freney and Wetselaar, 1967). With both laboratory methods the soil samples were extracted with 2M KCl, and with the distillation the nitrate was reduced to ammonia after first distilling over any extracted ammoniacal-N. For both of these methods a 5-g subsample of a soil sample was shaken with 25 mL of 2M KCl for one hour. After settling, 15 mL of the clear supernatant was used for distillation and 5 mL for the autoanalyzer. In addition, seven soil water extracts used for the reflectometer method were stored at 4°C for 2 days, after which their nitrate concentration was again determined using the reflectometer method.
FIGURE 1. Relation between the three different methods of determining nitrate concentrations in soil. The squares of the correlation coefficients ($r^2$) are 0.999 (A), 0.966 (B), and 0.957 (C).
FIELD MEASUREMENT OF SOIL NITRATE CONCENTRATIONS

TABLE 1. Soil nitrate concentrations of seven filtered soil extracts determined with the reflectometer immediately after extraction with water and after 2 days storage at 4°C.

<table>
<thead>
<tr>
<th>Soil sample no.</th>
<th>At day 1</th>
<th>At day 3</th>
<th>NO$_3^-$ (mg L$^-1$)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>31</td>
<td></td>
<td>25.1</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>58</td>
<td></td>
<td>58.5</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>11</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>26</td>
<td></td>
<td>26.0</td>
</tr>
<tr>
<td>11</td>
<td>33</td>
<td>37</td>
<td></td>
<td>35.0</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>9</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>6</td>
<td></td>
<td>6.0</td>
</tr>
</tbody>
</table>

There was excellent agreement between the distillation and autoanalyzer methods (Figure 1A), and the reflectometer method compared well with both of them (Figure 1B and C), all correlations being extremely high and significant. In addition, the storage of the water extracts for two days had no significant effect on their nitrate concentrations (Table 1).

The Effect of Temperature

Discrepancies were noticed between values of samples measured in the field and those of the same samples measured later under constant laboratory conditions. Because the field analyses were made at different ambient temperatures, it was suspected that temperature itself could affect the method. To investigate this, a series of standard solutions (of 25, 50, 100, 150, and 200 mg NO$_3^-$ L$^-1$), prepared at 25°C, was tested in constant-temperature rooms held at 6.6, 18.7, 24.5, 30.6, and 36.6°C, using two meters and a common batch of test strips. The nitrate concentrations were then determined in triplicate with each meter. An increase in temperature resulted in higher readings for a given nitrate concentration (Figure 2A). The discrepancies varied between an underestimate of 25% at 6.6°C to an overestimate of 80% at 36.6°C. Presumably, the discrepancies predominantly reflect the temperature dependence of the reductive step of the chemical reaction on the test strips, since the reflectance measurement is likely to be insensitive to temperature. Expressed in another way, the data indicated that for each meter the temperature at which the meter reading (MR) coincided with the true nitrate concentration (TNC) was highly dependent on the nitrate concentration of the solution (Figure 2B). For instance, for 25 mg NO$_3^-$ L$^-1$ MR=TNC at 20-20.5°C, while for 150 mg NO$_3^-$ L$^-1$ MR=TNC at 8-10°C.

Determination of Nitrate in Soil

Nitrate is normally extracted from soils by shaking a representative sample of known weight with a known volume of 2M KCl (Bremner and Keeney, 1966).
FIGURE 2. Top: Relation between nitrate true nitrate concentrations and readings given by the reflectometer (meter A) as a function of temperature. Bottom: Relation between the true nitrate value (TNV) and the temperature at which the reflectometer reading (MR) is equal to TNV, for two meters A and B.
FIGURE 3. Relation between actual nitrate concentration and meter reading as affected by KCl concentration at 25°C. The squares of the correlation coefficients \( r^2 \) are 0.967, 1.000, 0.999, and 0.999 in ascending order of KCl concentration.

The function of the KCl is to flocculate the clay and to inhibit denitrification during the period of extraction and settling, which normally takes several hours. After the soil particles settle, a clear supernatant solution is generally obtained, the nitrate concentration of which can be determined colorimetrically or by distillation. In the present study it was found that with the reflectometer method, KCl affects the detection of nitrate even at very low KCl concentrations (Figure 3).

In order to establish which ion was responsible for this effect a comparison was made, in triplicate at 25°C, of KCl, MgCl\(_2\), K\(_2\)SO\(_4\), and water solutions containing different nitrate concentrations. It was shown (Figure 4) that the interfering ion was Cl\(^-\) and that K\(_2\)SO\(_4\) gave the same readings for the different nitrate concentrations as water only.

Soil samples that differ widely in clay content (from 8 to 50%) were extracted with 0.5M K\(_2\)SO\(_4\). After 5 minutes of occasional shaking, followed by a settling period of about half an hour, clear filtrates could be obtained after filtering through a nitrate-free filter paper such as Whatman No. 30.
FIGURE 4. Relation between actual nitrate concentration and meter reading as affected by different salts at 25°C. The squares of the correlation coefficients ($r^2$) were all greater than 0.99.

**Soil Extraction with Water**

The above method is useful in the field when only a small amount of extract solution is used, for example when subsamples of about 5-10 g are sufficient. In general, however, it is desirable to avoid possible errors associated with measurements on a subsample that represents only a small proportion of the total sample. For example, if a farmer wants to assess the nitrate concentration of a 60-cm soil layer of his paddock, he would have to collect about 10 cores using a 5-cm diameter auger. This would result in a soil sample of approximately 15 kg. It would then be extremely difficult to obtain a 10 g subsample that could be regarded as representative. However, without much equipment, a 1-kg representative subsample can easily be obtained. Extracting such an amount of soil with 2 to 1 (v/w) extractant would require 2 L of extractant. Using a 0.5M $\text{K}_2\text{SO}_4$ solution for this purpose would be forbiddingly expensive. Consequently, a modified method was developed in which a clear soil extract is obtained using only water as the extractant and filtering a part of the supernatant through a 0.8-mm filter. The nitrate in the clear filtrate is then determined as described earlier.
In detail, nitrate-free water, which has also a low chloride concentration, is added to the soil subsample in the ratio 2 to 1 (v/w) extractant to soil. The slurry is vigorously stirred occasionally during the first 5 minutes, thereafter it is allowed to settle for a maximum of half an hour (any longer contact between soil and water might lead to nitrate losses through denitrification). In the meantime, a 25-mL syringe is connected to the top of a 26-mm diameter, 0.8-mm filter. Approximately 3 mL of the supernatant of the soil extract is poured into the top of the syringe, the plunger is inserted and pressed down to produce a minimum of 6 drops of filtrate in a collector tube with a minimum internal diameter of 7 mm.

Another subsample can be collected for determination of the soil moisture content to allow calculation of the nitrate concentration on a dry soil basis. In the field, the necessary facilities for soil drying are usually not available. Soil moisture content can to some accuracy be assessed by “feel,” accuracy increasing with experience. The effect of the error in estimating soil moisture content in this way on the error in the soil nitrate assessment is given in Figure 5, where it can be seen that (i) the lower the actual soil moisture percentage the lower the error in nitrate assessment and (ii) even a 40% error in moisture content assessment induces in most cases not more than a 20% error in the nitrate assessment.
DISCUSSION AND CONCLUSIONS

The Nitrachek/Merckoquant reflectometer method is an easy and quick method to determine the approximate nitrate concentration of water samples in the field. In view of the high sensitivity of the method to temperature (Figure 2) such readings can at best be used as a rough indicator. For an accurate result, it is recommended that an additional water sample is collected on which the nitrate concentration is determined under constant temperature conditions that allow for comparison with standard solutions.

The method can also be used readily to determine the nitrate concentration in soils. Extraction with KCl solution is not suitable due to the interference of the chloride ion with the reaction on the test strip. This can be overcome by extracting with 0.5M K₂SO₄ and filtering the supernatant through filter paper. Where large subsamples are obtained, however, it is cheaper to extract with water and filter the extract under pressure using a 0.8-mm cellulose acetate filter attached to a syringe. Laboratory tests and field experience with hundreds of soil samples suggest that even with soils that have a very high clay content sufficient extract can be obtained to activate the color pads.

In the water-extraction procedure recommended above for soil samples, the extraction time has been kept as short as possible to avoid a possible decrease of the nitrate level due to denitrification during extraction. The comparison of the reflectometer method with the other two methods (Figure 1) suggests that even with topsoils that are relatively high in organic matter (sample numbers 1, 6, and 11 in Table 1) nitrate loss due to denitrification after filtration does not take place with the recommended procedure. In addition, it appears that filtered soil extracts can be stored at low temperatures for at least two days.

The extraction of the soil with water rather than with K₂SO₄ allows for a very large subsample. This can be an advantage when the heterogeneity of the soil aggregates makes it difficult to obtain a representative subsample.

ACKNOWLEDGMENTS

We thank James Lavett for assisting with the measurements of the effect of different salts on the reflectometer readings. We are grateful to Ian Fillery and Lorraine Tonnet for helpful comments.

REFERENCES


