Mid-infrared Diffuse Reflectance Spectroscopy for the Quantitative Analysis of Agricultural Soils

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Soil samples were analyzed conventionally and by mid-infrared diffuse reflectance spectroscopy for total C, total N, pH, and measures of biological activity. Ground, non KBr diluted, samples (n = 180) from experimental plots (two locations, three replicate plots, under plow and no-till practices, three rates of N fertilizer, and from five depths) were scanned from 4000 to 400 cm^{-1} (4-cm^{-1} resolution, 64 co-added scans) on a DigiLab FTS-60 Fourier transform spectrometer using a custom-made linear sample transport (50 by 2 mm sample area scanned). Results using partial least-squares regression showed that accurate calibrations can be developed for the determination of a number of compositional parameters: total C, total N, pH, and many measures of biological activity. In general, the results achieved using mid-infrared spectra were at least as accurate as those found previously using near-infrared spectra and were sometimes significantly better, that is, pH.

Keywords: *Mid-infrared; DRIFTS; soil; quantitative analysis*

INTRODUCTION

Determination of soil composition can be time-consuming and expensive, especially when large numbers of analytes are involved. This is also true for many other areas of research and commerce, and thus over the past two decades spectroscopic methods have come to dominate many areas of analytical chemistry in which there is a need for rapid, inexpensive, and accurate determination of analytes. Predominately this has involved the utilization of spectroscopy in the visible and near-infrared (NIR) ranges (400−2498 nm) and has come to be known as near-infrared (NIR) spectroscopy or NIRS (*1*). When combined with regression analysis [multilinear, principal components, partial least-squares (PLS), etc.], NIRS has come to be used to determine the composition of a wide variety of materials ranging from animal feeds (*1*) and manures to foods (*2*) and pharmaceuticals (*3*). Once a calibration relating the spectra to the property of interest (i.e., fiber or crude protein content) is developed, new samples can be analyzed for a multitude of components (as many as one has calibrations for) in a few minutes without the need for time-consuming conventional methods. Recently, interest in the use of NIRS for soil analysis has greatly increased, and NIRS has been shown to be able to rapidly and accurately determine many components of interest including total N, organic C, etc. (*4*−*7*).

Although NIRS has developed into a major tool for analytical determinations over the past two decades, the same is not true of the mid-infrared (2500−25000 nm), which is used mainly for research or qualitative analysis involving spectral interpretation. The central reason for this has been the belief that quantitative analysis on powdered samples required sample dilution with spectral grade KBr or the preparation of KBr disks, etc. (*8*), due to the strong absorptions present in the mid-infrared region. These strong absorptions result in spectral distortions and nonlinearities (*9*), which were believed to make quantitative analysis difficult or impossible with "as is" (non KBr diluted) samples. However, work on a variety of products including foods (*10*−*12*), forages (*13*), and soils (*14*) has demonstrated that quantitative analysis using mid-infrared diffuse reflectance spectroscopy (DRIFTS) and "as is" (neat) samples can be performed with an accuracy equal to or greater than that achieved using NIRS.

Although NIRS is limited in its usefulness for qualitative analysis (spectral interpretation), due to the many overlapping bands and also because only bands due to OH, NH, or CH are present (*15*), the mid-infrared excels in such areas with entire books on the subject (*16*). In addition, bands exist for many soil constituents in the mid-infrared, which do not exist in the NIR, including those for inorganic C in the form of carbonates (*17*). Also, other minerals such as phosphates have spectral absorptions in the mid-infrared, but none in the near (*18*). Thus, the mid-infrared region potentially has advantages over the NIR for the analysis of soils, although considerable work is still needed to determine the effect of factors such as sample size (*19*), particle size (*20*, *21*), moisture content, etc., on mid-infrared calibrations. The objective of this work was to investigate the ability of DRIFTS to accurately determine the composition of soil samples from different depths, geographical locations, and tillage practices and with different rates of nitrogen fertilization.

MATERIALS AND METHODS

Samples. Soil samples were obtained from two locations in Maryland, the first (L1) consisted of a well-drained Delanco silt loam (Aquic Hapludult) from the Piedmont region near

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Table 1. Composition of Soils Used in Studies

assay[a]	mean	SD	minimum	maximum
total C[b]	13381	4636	6130	33900
total N[b]	1187	379	600	2770
pH[b]	6.38	0.65	4.40	7.30
active N[b]	165.9	82.0	66.0	513.2
biomass N[b]	90.6	42.5	20.2	251.6
mineralizable N[b]	10.6	5.86	0.20	33.50
arylsulfatase[c,d]	62.0	48.5	13.4	380.2
dehydrogenase[c,e]	10.21	6.71	0.20	37.8
nitrification potential[c,f]	9.54	6.07	2.10	32.0
phosphatase[c,d]	310.8	123.0	126.4	670.5
urease[c,g]	1.86	0.90	0.60	5.40

[a] Total C, total N, active N, and biomass N in milligrams per kilogram of dried soil. [b] $n = 180$. [c] $n = 174$ due to removal of six concentration outliers. [d] Activities as micrograms of *p*-nitrophenol produced per gram of soil per hour. [e] Activity as micrograms of *p*-triphenylformazan produced per gram of soil per hour. [f] Activity as micrograms of nitrate N produced per gram of soil per hour. [g] Activity as picograms of urea hydrolyzed per gram of soil per hour.

Clarksville, MD, and the second (L2) a somewhat poorly drained Bertie silt loam (Aeric Endoacquult) from the Delmarva Pennisula near Queenstown, MD. These sites were long-term field (maize under cultivation) experiments (>20 years) with both no-till and plow-tillage plots present at each site (*22*). At each location samples were obtained as follows: two tillages, five depths (0−2.5, 2.5−5.0, 5.0−7.5, 7.5−12.5, and 12.5−20 cm), three different rates of N fertilization (0, 135, and 270 kg of N ha^{-1}), and three replicate plots for each condition, for a total of 180 samples ($2 \times 2 \times 5 \times 3 \times 3$). Samples were obtained in June ~2 months after tillage had occurred (April). All samples were sieved "as is" (field moist) to pass a 4-mm screen and mixed, and subsamples were taken for analysis.

Conventional Analysis. Total C and total N were determined on samples dried at 105 °C for 24 h using a Leco CNS-2000 elemental analyzer (Leco Corp., St. Joseph, MI). All other analyses were performed on field moist samples (as is). Active N was determined by ^{15}N isotope dilution (*23*), biomass N by the fumigation−incubation technique (*24*), and mineralizable N as the soil N mineralized during a 21-day aerobic incubation at 25 °C (*25*). Soil pH was determined by measuring the pH in a 2:1 water/soil slurry. Further details on the samples, nature of the experimental plots, and procedures used to determine the various soil constituents may be found in McCarty and Meisinger (*22*).

Mid-infrared Spectroscopy. Samples (105 °C dried samples) were scanned in the mid-infrared from 4000 to 400 cm^{-1} (2500−25000 nm) at 4-cm^{-1} resolution with 64 co-added scans per spectra, on a DigiLab FTS-60 Fourier transform spectrometer equipped with a custom-made sample transport that allowed a 50 by 2 mm area sample to be scanned (*19*). Absorbance spectra were collected as log(1/reflectance) using KBr for the background reference.

Regression Analysis. Descriptive statistics (Table 1) were performed using SAS (*26*). All analysis of DRIFTS data was performed by PLS using Grams/386 PLSPlus V2.1G (*27*). Efforts using a variety of data subsets, spectral data point averaging, derivatives (first and second), and other data pretreatments (mean centering, variance scaling, multiplicative scatter correction, and baseline correction) were carried out to determine the best data pretreatment for each assay. In all cases, the number of PLS factors used in the calibration was determined by the prediction residual error sum of squares (PRESS) statistic from the one-out cross-validation procedure. Once the optimal number of PLS factors was determined, a final calibration was developed. For discriminant analysis of tillage and location, values of 1 and −1 were used and PLS calibrations developed as above. Although three replicate plots were used for the original experiments (*22*), there was substantial spatial variability, and therefore replicate soil samples varied greatly in properties. On this basis, the one-out cross-validation analysis was the most reasonable measure of error associated with soil property estimates. The same one-out analysis was also preformed in the NIR, as discussed below, thus allowing direct comparisons of the results achieved using the two spectral regions.

Table 2. Some Compositional Determinations of Soils As Influenced by Tillage

assay	mean	SD	minimum	maximum
Plow-Till Samples ($n = 90$)				
pH	6.33	0.61	4.80	7.30
total C	11795	1640	6670	15200
total N	1040	141	610	1340
active N	132	27.6	66.0	230
biomass N	76.9	16.4	34.9	109
No-Till Samples ($n = 90$)				
pH	6.43	0.69	4.40	7.20
total C	14967	5955	6130	33900
total N	1334	474	600	2770
active N	199	103	67.6	513
biomass N	104	54.6	20.2	252

Near-infrared Studies. It should be noted that the same samples were previously scanned in the near-infrared spectral region from 400 to 2498 nm using a scanning monochromator (*4*, *28*). The same analyte determinations were also used for both studies. As such, the only difference between the results presented here and the previous studies is that of the spectral range involved.

RESULTS AND DISCUSSION

Samples. As can be seen from the data in Table 1, a wide variety of samples from both agronomic and compositional standpoints were available for study. Concentrations of analytes varied 5-fold or more for all analytes except pH. In addition, as discussed under Materials and Methods, the samples varied widely in agronomic factors such as source of soil (location and depth), farming practice (plowed and no-tilled plots), and rate of fertilization. As might be expected, the composition of the samples also varied greatly due to factors such as tillage (Table 2), depth, location, etc. (not shown). These factors combine to result in a very diverse sample set in which both the biological and chemical compositions of the soils vary across the data set, thus presenting a number of factors that could be examined for effect on calibration accuracy. Although not demonstrated directly by the data in Table 2, it is also to be expected that differences in the forms of organic matter present occur due to tillage practice. Thus, the much higher maximum values shown for total C, etc., with the no-till samples are due to the presence of large amounts of surface residues. It is also to be expected that the composition of these surface residues will be different from the organic matter found in the soil itself where humification has proceeded to a greater degree (*22*). One would also expect differences in the forms of organic matter present deep within the soils due to the tillage practice, with the no-till organic matter being derived primarily from decaying roots or migration of soluble material from the surface, whereas the tilled soils would also contain the products resulting from the decay of nonsoluble surface residue material that has been plowed under. The result is that spectroscopic calibrations have to handle not only quantitative differences but also qualitative differences in the forms of organic C, N, etc. present.

Spectra. In Figures 1 and 2, the mid-infrared and NIR spectra of the samples with the greatest and least concentrations of C are shown. The highest C sample

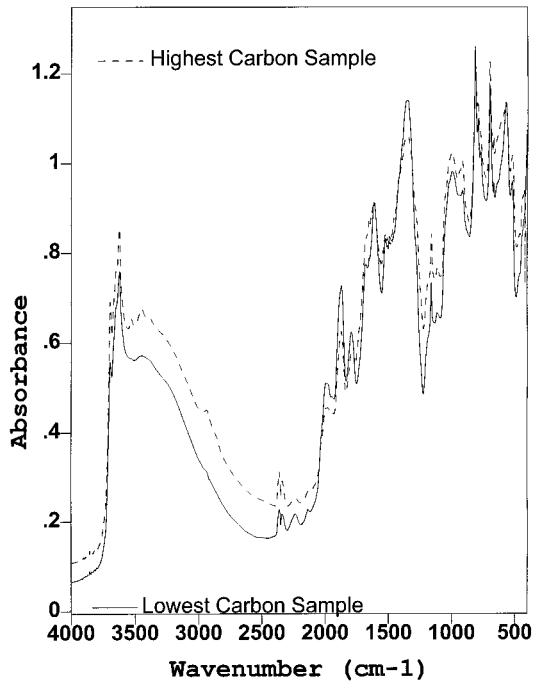

Figure 1. Mid-infrared spectra of soil samples with highest and lowest carbon contents (n = 180).

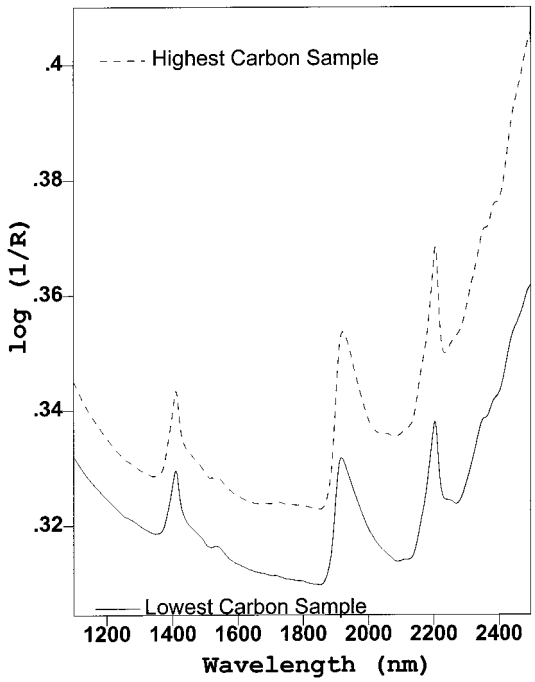

Figure 2. Near-infrared spectra of soil samples with highest and lowest carbon contents (n = 180).

was from the surface of a no-till plot and the lowest from the 12.5−20 cm depth of a no-till plot. Comparing the mid-infrared (Figure 1) and NIR (Figure 2), one sees that the mid-infrared spectra contain definable peaks, which could be used in spectral interpretation and at least some differences between the two samples. By contrast, the NIR spectra appear to be virtually identical except for the baseline shift, which is likely due to particle size differences and not compositional factors.

Calibration Results. *All 180 Samples.* The PLS results achieved using all 180 samples are shown in Table 3. As can be seen, the results for location, total C, and total N were excellent on the basis of either the one-out cross-validation or the final calibration. The final calibration results for total N are shown in Figure 3. As shown, the overall results were quite good with few if any samples poorly determined, although more samples at the higher levels (≥2000 mg/kg) might be desirable for a more complete calibration. Of the non-compositional parameters (location, tillage, sample depth, and N rate), only location was completely discriminated. However, the results for tillage were close to complete discrimination (Figure 4). Using NIR spectra (*4*), accurate discrimination was achieved only for location.

Table 3. One-Out Cross-Validation and Final Calibration Results Using All Usable Samples (n= 180 or 175, See Table 1)

	one-out cross-validation results			calibration results		
assay[a]	factors	R^2	RMSD[b]	R^2	RMSD	RMSD (%)/mean
location	15	0.993	0.0816	0.996	0.061	
tillage	14	0.731	0.527	0.872	0.358	
N rate	13	0.549	75.1	0.685	61.9	45.9
depth	12	0.538	1.44	0.690	1.17	39.0
pH	14	0.911	0.194	0.940	0.159	2.5
total C	13	0.957	963	0.976	713	5.3
total N	13	0.955	80.5	0.971	63.9	5.4
active N	7	0.869	29.6	0.901	25.7	15.5
biomass N	5	0.790	19.4	0.829	17.5	19.3
mineralizable N	2	0.150	5.39	0.174	5.31	50.1
arylsulfatase	9	0.682	14.1	0.720	13.2	23.4
dehydrogenase	5	0.656	3.85	0.722	3.46	34.3
nitrification potential	8	0.725	2.90	0.771	2.26	24.5
phosphatase	7	0.797	56.7	0.828	52.2	16.7
urease	13	0.740	0.427	0.831	0.343	18.7

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$.

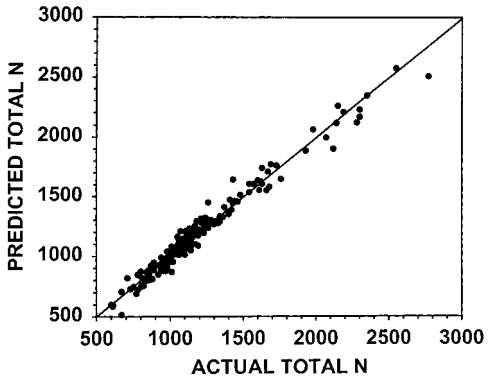

Figure 3. Final calibration results for total N using mid-infrared spectra (n = 180).

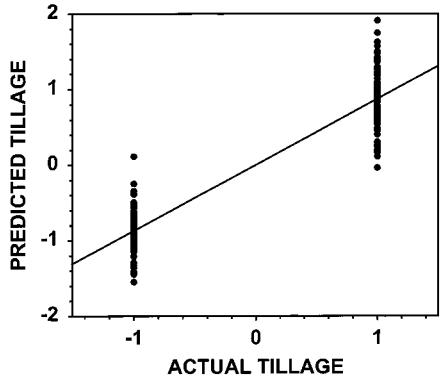

Figure 4. Final calibration results for discrimination of tillage format using mid-infrared spectra (n = 180).

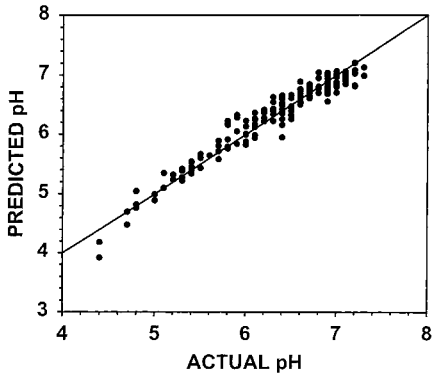

Figure 5. Final calibration results for pH using mid-infrared spectra ($n = 180$).

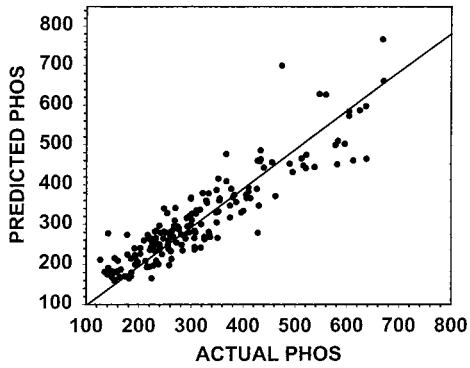

Figure 6. Final calibration results for phosphatase enzyme using mid-infrared spectra ($n = 175$).

Likewise, the mid-infrared results for pH (Figure 5) were quite good ($R^2 = 0.940$) and much better than achieved using NIR spectra ($R^2 = 0.874$; *4*). Overall, the mid-infrared results for total C, total N, and active N were somewhat better than achieved using NIR spectra, and those for biomass N and mineralizable N were about the same. Also, although the results for active and biomass N are not as good as might be desired, the nature of the assays (biological assays with greater variability than conventional chemical assays) could easily be the reason. Unfortunately, neither spectral region was useful in determining mineralizable N, a factor of great interest and importance.

Finally, while the results for the various enzyme activities would not support their replacement by spectroscopic calibration, the results achieved were somewhat better than achieved using NIR spectra (*28*) and indicate that further efforts are needed to determine the limitation of such spectroscopic based calibrations. Again, these are biological assays with high inherent variability as indicated by the removal of five samples due to obviously bad analyte determinations (*28*). Also, examination of the data indicates that more analyte values might be bad as shown in Figure 6 for phosphatase. The removal of a few samples would result in a calibration acceptable at least for differentiating high and low activities. In summary, the results for a variety of analytes using mid-infrared spectra were overall better than those achieved using NIR spectra and were never worse.

Using Two-Thirds of Samples as a Calibration Set. Although the results presented in Table 3 indicate that it is possible to develop accurate and useful calibrations to determine a wide variety of analytes and soil parameters using mid-infrared spectra, the question remains as to what happens when calibrations developed using one set of samples are applied to another set (how robust are the calibrations?). For the results in Table 4, the samples were split into a calibration set consisting of two-thirds of the samples and a validation set consisting of the remaining one-third. These samples were chosen by randomly selecting samples from subsets of the 180 samples. Samples were divided into subsets consisting of location, tillage, and N rate for a total of 12 subsets of 15 from which 5 samples from each set of 15 were randomly chosen as validation or test samples. Except for tillage, the results indicate that the development of robust calibrations should be possible. Although the errors [root-mean-squared deviation (RMSD) or RMSD/mean] generally increased, the increases were not great considering the diverse nature of the samples. The results for pH showed one of the greater decreases in performance and indicate that mineralogy, as reflected by location, of the samples may be an important factor; because each sample came from a different plot, depth, location, etc., the diversity was difficult to overcome. However, as shown in Figure 7, the results might still be acceptable for a quick determination or when thousands of samples might be involved. Also, the results for the various enzymes appear to be more robust, although obviously not as accurate, as, for example, those for total C.

Comparing these results with those based on NIR spectra showed some calibrations to be more robust and some less. In particular, the mid-infrared-based calibra-

Table 4. Results Using Two-Thirds of Samples as a Calibration Set and the Remaining One-Third as a Validation or Test Set

	calibration from one-out cross-validation ($n = 120$)			validation results ($n = 60$)		
assay[a]	factors	R^2	RMSD[b]	R^2	RMSD	RMSD (%)/mean
location	14	0.997	0.0574	0.988	0.115	
tillage	11	0.864	0.368	0.350	0.964	
pH	14	0.950	0.153	0.833	0.228	3.5
total C	13	0.978	670	0.931	1253	9.2
total N	12	0.971	64.0	0.951	79.1	6.6
active N	6	0.874	29.0	0.917	24.7	14.5
biomass N	4	0.779	20.2	0.817	18.0	18.9
arylsulfatase	7	0.674	14.7	0.659	13.8	23.5
dehydrogenase	4	0.661	3.72	0.668	3.97	38.0
nitrification potential	6	0.736	2.75	0.637	3.56	37.2
phosphatase	5	0.818	56.8	0.698	60.6	20.3
urease	13	0.827	0.317	0.789	0.458	23.8

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$.

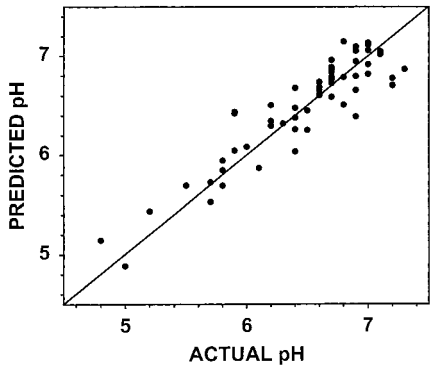

Figure 7. Validation set results ($n = 60$) for determination of pH using calibration based on 120 samples.

Table 5. Calibration Results Using Only Plow-Tilled or No-Tilled Samples

	plow-till			no-till		
assay[a]	factors	R^2	RMSD[b]	factors	R^2	RMSD
location	15	0.997	0.0504	14	0.998	0.0498
pH	12	0.926	0.166	11	0.956	0.143
total C	7	0.943	390	14	0.988	657
total N	7	0.924	38.6	13	0.985	57.4
active N	5	0.657	16.1	4	0.896	32.9
biomass N	5	0.650	9.64	4	0.853	20.8
arylsulfatase	12	0.674	14.7	7	0.642	15.8
dehydrogenase	3	0.607	2.94	4	0.720	4.20
nitrification potential	8	0.818	1.24	6	0.767	3.31
phosphatase	7	0.820	44.9	4	0.825	60.2
urease	13	0.788	0.196	7	0.793	0.469

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$.

tions for pH and total N were considerably better than those achieved using NIR spectra, whereas total C was slightly better using NIR spectra, and the results for location and active and biomass N were about the same for the two spectral regions. Overall, the calibrations for the mid-infrared and NIR do not appear to be significantly different with respect to the question of robustness, although the variations in how the various analytes performed using the two regions may indicate some differences in the basis for the determinations. For example, minerals do not have spectra in the NIR (*18*) but do in the mid-infrared (*29*). Thus, the better results achieved in the mid-infrared for pH may be due to the ability of the mid-infrared to base the calibration on mineral differences not apparent in the NIR spectra.

Tillage-Based Calibrations. As previously discussed, it would be expected that the no-till and plow-tilled samples would possess not only different levels of organic material but also different forms, due to the presence of surface residue in the no-till plots and the percolation of soluble materials in the decomposing surface residue into the soil with rain. These differences could have significant impacts on the ability to develop calibrations or on their robustness. The results in Tables 5 and 6 are for calibrations developed using only one type of sample (Table 5) or for predicting one type with a calibration from the other. The data in Table 5 demonstrate that it is possible to develop accurate calibrations using either set of samples alone. Except for the active and biomass N determinations, the differences in the calibrations between the two sets are relatively minor and most likely due to the different ranges of the analyte values present. For example, the RMSD for the no-till samples are generally higher, but then so are the average analyte values themselves (Table 2). Also, the markedly lower R^2 for active and biomass N may be more a reflection of the narrow range of values in the plow-tilled samples than anything else.

The one dramatic difference between the two sets of calibrations and also between these and the calibrations using all of the samples (Table 3) is the number of calibration factors required for many of the analytes. For total C and N, the number of factors required for the plow-till calibrations was only half the number used for the no-till calibrations or in the all-sample calibration (Table 3). This is most likely a reflection of the relative diversity in the composition of the organic matter present in the two sample sets as previously discussed (*22*).

Table 6. Results of Determining Plow-Tilled or No-Tilled Samples with Calibration and Modified Calibrations Developed Using the Other Set of Samples

	no-till by plow-tilled			plow-tilled by no-till		
assay[a]	R^2	RMSD[b]	bias[c]	R^2	RMSD	bias
A. Calibration						
location	0.969	0.264	0.155	0.926	0.288	−0.084
pH	0.875	0.363	−0.073	0.621	0.451	0.245
total C	0.906	3058	−991	0.701	1792	968
total N	0.929	232	−119	0.662	163	89
active N	0.659	79.9	−37.6	0.295	32.9	18.9
biomass N	0.738	35.5	−10.5	0.484	16.4	−1.7
arylsulfatase	0.585	18.6	−6.5	0.312	28.7	20.5
dehydrogenase	0.386	6.58	−1.42	0.646	3.03	−0.98
nitrification potential	0.628	4.73	−1.66	0.497	2.45	1.15
phosphatase	0.809	82.0	18.6	0.669	67.0	−24.8
urease	0.676	0.648	−0.271	0.346	0.560	0.341
B. Modified Calibrations[d]						
location	0.998	0.136	0.058	0.953	0.238	−0.065
pH	0.912	0.268	0.120	0.833	0.277	0.120
total C	0.922	1669	92.2	0.802	1388	763
total N	0.922	138	−47.6	0.824	107	55.6
active N	0.810	46.3	−17.4	0.318	32.0	16.1
biomass N	0.750	28.1	−3.18	0.490	15.9	−2.65
arylsulfatase	0.581	20.2	−5.99	0.287	29.2	19.9
dehydrogenase	0.550	5.22	0.333	0.275	5.41	−2.05
nitrification potential	0.735	3.70	−1.41	0.466	2.31	0.559
phosphatase	0.821	61.3	0.52	0.687	67.9	−28.4
urease	0.686	0.653	−0.188	0.408	0.423	0.157

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$. [c] Bias = difference in predicted and actual mean value. [d] Eight samples removed from set to be predicted, added to calibration set, and new calibration developed.

When the calibrations developed using one set of samples were used to determine the other, the results were almost always better using the plow-tilled calibration to determine the composition of the no-till samples. Because the no-till samples contain a wider range of values, due to surface residue and stratification of organic matter with depth, and also contain many subsurface samples, which one would assume are similar in composition to the plow-tilled samples, logic would dictate that the no-till calibration should be the better and more robust calibration, but this was not the case. The same results were found in the NIR study (*4*). No easy explanation exists at present to explain these results. Because calibrations are based on the chemical composition of the analytes in question, it would appear that the surface residue present in the no-till samples results in a calibration based on compositional parameters which nonsurface organic matter lacks but that the opposite is not true. Only further investigations using other techniques to determine the nature of the organic matter present in each type of sample and relating this composition data to the basis for NIR and mid-infrared calibrations can provide the answer.

Results of efforts to improve the ability of the plow-till or no-till calibrations to determine their counterpart samples are shown in Table 6B. Eight samples were chosen on the basis of extremes in tillage (plow-till and no-till), depth (0−2.5 and 12.5−20 cm), and N rate (0 and 270 kg of N ha^{-1}) for each location and added to the original sample set, and a new calibration was developed. As can be seen, adding a few samples from one set to the calibration of the other and developing a new calibration greatly improved the ability of the tillage-based calibrations to determine their counterpart

Table 7. Results of Calibrations Based on Samples from One Location Only

	location 1			location 2		
assay[a]	factors	R^2	RMSD[b]	factors	R^2	RMSD
tillage	10	0.888	0.334	12	0.944	0.237
pH	13	0.964	0.139	13	0.898	0.136
total C	7	0.956	917	10	0.981	652
total N	11	0.971	62.0	6	0.967	67.7
active N	8	0.928	21.8	5	0.935	20.8
biomass N	8	0.894	13.8	5	0.900	12.9
arylsulfatase	9	0.649	15.5	6	0.803	9.01
dehydrogenase	4	0.542	4.36	3	0.752	2.29
nitrification potential	13	0.857	1.78	6	0.828	2.52
phosphatase	5	0.800	55.0	4	0.838	46.6
urease	10	0.747	0.338	12	0.910	0.288

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$.

Table 8. Results of Determining Samples from One Location with Calibration Developed Using Samples from Another Location

	L1 by L2			L2 by L1		
assay[a]	R^2	RMSD[b]	bias[c]	R^2	RMSD	bias
tillage	0.237	6.73	6.58	0.134	4.44	4.28
pH	0.784	0.770	0.666	0.338	0.935	−0.849
total C	0.881	3391	2989	0.949	1254	−390.3
total N	0.924	121	65.9	0.946	179	−49.3
active N	0.777	48.7	29.3	0.893	121	−117
biomass N	0.665	84.0	80.3	0.763	30.6	−21.9
arylsulfatase	0.374	24.4	−12.2	0.546	68.8	−66.4
dehydrogenase	0.303	12.4	−11.2	0.692	14.4	14.1
nitrification potential	0.470	5.55	3.86	0.688	12.2	11.1
phosphatase	0.598	231	−209	0.734	84.0	−52.6
urease	0.094	1.95	1.79	0.616	1.26	−1.11

[a] See Table 1 for definitions of total C, N, etc.; N rate = rate of application of NH_4NO_3 fertilizer. [b] Root-mean-squared deviation = (sum squared residuals/N)$^{1/2}$. [c] Bias = difference in predicted and actual mean value.

samples for some analytes, but not for others, and generally performed better when starting with the plow-tilled calibration, especially for the biological measures that is, active and biomass N and the enzymes (some plow-tilled measures were determined less accurately by the modified calibrations than by the nonmodified). Although these results show that mid-infrared-based calibrations behave in a manner similar to NIR-based calibrations and that calibrations can be improved by adding new samples to old calibrations to increase the robustness of the calibration, the wide variation in the results achieved indicates that further studies with larger sample sets will be needed to fully determine the factors influencing the robustness of mid-infrared soil calibrations, especially for measures of biological activity.

Location-Based Calibrations. In developing and using calibrations, it is likely that calibrations based on samples from one location should be used to determine new samples taken from other locations. In Tables 7 and 8, the results are shown for calibrations developed using samples from each location separately and for determining samples from one location with a calibration developed using samples from a second location. As can be seen, using samples from only one location gave fairly similar results for either location, although there were some significant variations for some of the enzymes (arylsulfatase, dehydrogenase, and urease). From a comparison of these results with the earlier NIR study (*4*), only one real difference stood out and that was the results for tillage. In the NIR, tillage was never discriminable, but the results here, and also those in Table 3, indicate that the mid-infrared calibrations were able to find some difference in the samples altered by tillage practice. Because this would most likely be related to differences in the nature of the organic matter present, these results indicate that the mid-infrared may be more able to differentiate subtle differences in organic matter composition than is the NIR.

Results of determining samples from one location with a calibration developed using samples from another location showed that determinations for total C and N, pH, and active N performed much better than similar NIR calibrations, suggesting that mid-infrared calibrations may be less affected by mineralogy (as reflected by location) than are NIR calibrations. This is somewhat surprising because, as previously discussed, minerals have a much greater spectral signature in the mid-infrared. The two soils involved were quite similar, so the question of differences in mineralogy may seem to be irrelevant, but although both were clays, the Clarksville site had considerable non-weathered, clearly visible mica, not present at the other site. Finally, although the results for pH were better than achieved with NIR calibrations, the results, especially for location 2 by location 1, and the mixed results achieved for the enzyme determinations would seem to indicate that the basis for these determinations may lie in the mineral composition of the soils and not on a direct determination of some organic fraction.

Conclusions. Results using 180 soil samples from two locations, with plots under plow-till and no-till practices, and three levels of nitrogen fertilization, with samples taken from five depths, showed that calibrations can be developed using mid-infrared spectra for the accurate determination of a number of compositional parameters including total C, total N, pH, and many measures of biological activity as reflected by enzyme activities and measures of biologically active N. In general, the results achieved using mid-infrared spectra were at least as accurate as those found previously using NIR spectra and were sometimes significantly better, that is, pH. Although efforts at determining the robustness of mid-infrared calibrations indicated that mid-infrared soil calibrations generally perform in a manner similar to NIR calibrations, differences found indicate that the basis for mid-infrared calibrations may at times be different.

ABBREVIATIONS USED

DRIFTS, mid-infrared diffuse reflectance spectroscopy; NIR, near-infrared; NIRS, near-infrared spectroscopy; PLS, partial least-squares regression; PRESS, predicted residual sum of squares; RMSD, root-mean-squared deviation; L1, location 1; L2, location 2.

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