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Sensitivity of Nitrogen Mineralization Indicators to Crop and Soil Management

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Abstract: Soil nitrogen (N) mineralization indicators are useful only if they are sensitive to management practices. The precision of measurement and the sensitivity of the following indicators to crop sequence, tillage, and liming effects were compared: (i) mineral N production during a 24-day incubation under aerobic conditions, (ii) ammonium (NH_4)-N production under waterlogged conditions, (iii and iv) hot potassium chloride (KCl)–extractable and hydrolyzable NH_4-N (the latter obtained by subtracting initial NH_4-N from extracted NH_4-N), and (v) protease activity. The coefficients of variation decreased in this order: protease activity > KCl-hydrolyzable NH_4-N > aerobic incubation > KCl-extractable NH_4-N = anaerobic incubation. Most of the test results obtained using the indicators were correlated with each other. Mineralizable N measured by aerobic and anaerobic incubation was sensitive to tillage, liming, and crop sequences, especially when using soil 5-cm deep. Hot KCl-extractable NH_4-N was influenced by tillage but not liming, and less sensitive than the incubation procedures to crop sequence. The protease assay produced no significant test. It was concluded that anaerobic incubation can provide a relatively sensitive assessment of management effects on soil mineralizable N.

Keywords: Anaerobic incubation, chemical index, nitrogen mineralization, protease activity

INTRODUCTION

There is continuing interest in soil nitrogen (N) mineralization because of its contribution to soil N supply, because of the potential for nitrate leaching

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during the off-season, and as an attribute of soil quality. The third aspect of N mineralization has not been as well researched and documented as the other two and is the object of this report. Soil-quality assessment can be useful for determining the sustainability of farming systems. Potentially mineralizable N, as evaluated by anaerobic soil incubation, is listed as one of the key biological indicators of soil quality by Doran and Parkin (1994). They suggested that soil-quality indicators must be sensitive to variations in management and climate and integrate soil properties and processes. Management practices such as cropping sequence and tillage intensity have been shown to influence N mineralization. For example, N mineralization increased as soil tillage intensity decreased (El-Haris et al. 1983; Kandeler, Tscherko, and Spiegel 1999; Soon, Clayton, and Rice 2001) and was higher when the preceding crop was a legume rather than a cereal crop (Francis, Hayes, and Williams 1994; Soon, Clayton, and Rice 2001).

Aerobic incubation of soil has long been used as the standard method to estimate potentially mineralizable N (Stanford and Smith 1972) and for assessing the effects of management practices on soil organic matter quality (Carter and Rennie 1982; Biederbeck, Campbell, and Zentner 1984). The method requires the maintenance of optimum soil water content during incubation and is time-consuming: incubation periods have varied from 2 to 30 weeks (Gasser 1961; Stanford and Smith 1972; Wang et al. 2001). Another method measures ammonium (NH_4)-N released during a 7-day incubation of soil under waterlogged conditions at 40°C. This is a more standardized, simpler, and quicker procedure than the aerobic incubation procedure. Ross (1997) reported that soil protease activity was significantly correlated to N mineralization in pasture soils. Protease catalyzes the hydrolysis of proteins to amino acids early in soil N mineralization process (Ladd and Butler 1972). Other studies also found correlations between both protease activity and N mineralization on one hand and enhanced protease activity in soil that received reduced or no tillage on the other (Kandeler, Tscherko, and Spiegel 1999; Zaman et al. 1999). Chemical methods of assessing N-mineralization potential are generally appealing because they usually are rapid and simple tests and more precise than biological tests. Øien and Selmer-Olsen (1980) and Whitehead (1981) proposed using hot potassium chloride (KCl)–extractable N as an index of available N on the basis of a close correlation with plant N uptake. Hot KCl-extracted NH_4-N was well correlated with crop N uptake and mineral N in the soil at sowing (McTaggart and Smith 1993) and with inorganic N released by anaerobic and aerobic incubations (Gianello and Bremner 1986b; Campbell et al. 1997). Jalil et al. (1996) found that mineralizable N was better correlated with hot KCl-extractable NH_4-N than with phosphate–borate–extractable NH_4-N (a quick chemical method for assessing soil N mineralization potential proposed by Gianello and Bremner, 1988). The simultaneous comparison of a wider selection of N mineralization indicators for their sensitivity to management practices has not been adequately studied.

The objective of this study was to evaluate N released by anaerobic incubation, protease activity assay, and hot KCl-extractable NH_4-N (with and without correction for initial NH_4-N) and compare them to aerobic incubation of soil for sensitivity to the effects of crop and soil management on mineralizable N. There were two parts to this study. First, the precision of measurement, using the coefficient of variation, the N mineralization indicators, the correlations among them, and some chemical properties of 23 farm soil samples were determined. Then, the sensitivity of the indicators to management practices using soils from two field studies that had shown differences in N availability and/or mineralization as a result of tillage, liming, and cropping systems effects (Soon, Clayton, and Rice 2001; Soon and Arshad 2005) were assessed.

MATERIALS AND METHODS

Twenty-three soils were collected from commercial farm fields in the Peace River region of northern Alberta to determine correlations between the N-mineralization indicators and their ability to detect differences among soils. The soils comprised 17 samples from the plow layer (normally 0–15 cm) and 6 samples from the 15- to 30-cm layer. The subsoil samples were included so as to have a wide range in soil organic matter and biological activity of the experimental soils. The soils were air dried, sieved (2 mm), and stored at room temperature for further analysis. Analysis of the soils was run in duplicate except for soil pH and organic carbon (C).

One field experiment was situated on a sandy loam soil with a pH of 5.7 and organic C content of 25 g kg^{-1} in the surface 15 cm. Experimental treatments were composed of factorial combinations of four rotations: pea (*Pisum sativum* L.)–wheat (*Triticum aestivum* L.)–canola (*Brassica rapa* L.)–wheat (PWCW), red clover (*Trifolium repens* L.)–wheat–canola–wheat (underseeded to red clover) (RcWCW), fallow–wheat–canola–wheat (FWCW), and continuous wheat (cW), and two tillage treatments: conventional tillage (CT) and no tillage (NT). Each treatment was replicated three times. Further details of the site and experiment are described in Soon, Clayton, and Rice (2001). Samples were obtained in autumn of 2000, 8 years after the experiment was initiated, from the 0- to 5- and 0- to 15-cm depths of the rotation plots only (i.e., excluded cW). Soil was sampled from the third and fourth phases of rotations to minimize the effects resulting from the different first phases of the rotations. The soil samples were air dried prior to measurement. Soils are normally air dried to facilitate handling and storage. Although air drying can affect soil microorganisms involved in N mineralization, it has been recommended by Campbell, Ellert, and Jame (1993) as a standardized procedure. In spring 2002, a second subset of soil samples was taken and not dried prior to the incubation studies to assess the influence of not drying soils before measurements. This sampling included rotation plots that had

previously grown canola, field pea, or red clover, as well as cW plots, so that the effects of preceding legume crops could be assessed.

The second experiment was located on a Hythe clay loam soil. The surface soil (0–15 cm) initially had a pH of 5.1 and contained 32 g kg^{-1} of organic C. The experiment was a 2 × 2 factorial, randomized complete block design with four replications to study the effect of lime (nil vs. 7.5 Mg ha^{-1}) and tillage practices (NT vs. CT) on crop production. In the autumn preceding the first experimental crop, lime was broadcasted and incorporated with a rotovator to a depth not exceeding 10 cm. The NT plots received no further tillage operations. Soil samples were taken (to 10-cm depth) in autumn of 2001, 10 years after the experiment was initiated, from all phases of the crop rotation (pea–barley (*Hordeum vulgare* L.)–canola). Then, the pH was 6.0 and 5.3 for limed and unlimed soils. The samples were air dried and passed through a 2-mm sieve.

Laboratory Nitrogen-Mineralization Indicators

Aerobic Mineralizable N

Nitrogen mineralization was determined by measuring the inorganic N [nitrate (NO_3)-N + nitrite (NO_2)-N + NH_4-N] produced during a 24-d incubation at 25°C in a growth chamber without light (Franzluebbers 1999). Soil moisture content was maintained at 80% of field capacity by periodic weighing. Containers were covered with Saran wrap® with two pin holes for gas exchange. Inorganic N was extracted by shaking a subsample of moist soil (equivalent to 5 g dry weight) at the end of incubation with 50 mL of 1 M KCl for 1 h.

Anaerobic Mineralizable N

In this method, N mineralization was estimated from NH_4-N produced during a 7-d waterlogged incubation at 40°C (Keeney and Bremner 1966). Five g of soil and 10 mL of nanopure water were incubated in a 16 by 150-mm screw-cap culture tube. After 7 d, the contents were quantitatively transferred to a 100-mL centrifuge tube using four washings of 10 mL of 1.25 M KCl to attain a final volume of 50 mL of 1 M KCl. After shaking for 30 min, the contents were passed through a Whatman no. 42 filter and analyzed for NH_4-N as described later. Initial NH_4-N content was subtracted from the result.

Hot 2 M Potassium Chloride–Extractable and Hydrolyzable NH_4-N

Three g of soil and 20 ml of 2 M KCl were transferred in 100-mL digestion tube, and the tube was stoppered and placed in a preheated (100°C) aluminum

digestion block (Gianello and Bremner 1986b). After 4 h of heating, the tubes were removed and allowed to cool, and 20 mL of distilled water was added to each tube. After mixing, the contents were filtered through a Whatman no. 42 filter. Extracted NH_4-N was determined to yield hot KCl-extractable NH_4-N. Recently, Jalil et al. (1996) reported that correlation with mineralizable N was better if the initial NH_4-N content was not subtracted from hot KCl-extractable NH_4-N. The procedure was originally designed by Gianello and Bremner (1986a), to assess potentially available organic N and initial NH_4-N content was subtracted from total extracted NH_4-N to give hydrolyzable NH_4-N. Both hot KCl-extractable and hydrolyzable NH_4-N were assessed.

Protease Activity

Protease activity was determined, with minor modifications, following Ladd and Butler (1972) and Speir et al. (1980). Five-g dry soil samples were moistened to field capacity and kept at room temperature for 1 week prior to analysis. Duplicate 0.4-g moist samples were incubated with 2 mL of Tris buffer [2-amino-2(hydroxymethyl)propane-1:3 diol, 2 M, pH 8.1] and 2 mL of sodium caseinate solution (2% w/v in Tris) at 40°C for 2 h in screw-cap culture tubes. A control without sodium caseinate was also incubated. After incubation, samples and controls were quickly brought to room temperature by immersion in cold water, and enzyme activity was stopped by adding 2 mL of trichloroacetic acid (15% w/v in H_2O). To the control, 2 mL of sodium caseinate solution was also added. After centrifuging, 1 mL of the supernatant solution was treated with 1.4 M disodium carbonate (Na_2CO_3) and a ninhydrin reagent, and absorbance was measured at 700 nm with a spectrophotometer. The control value was subtracted from the sample absorbance value for each soil. Results are expressed as nmol tyrosine equivalent g^{-1} soil h^{-1}.

Soil and Data Analysis

Total N (TN) was determined by a semimicro Kjeldahl digestion using copper sulfate/potassium sulfate ($CuSO_4/K_2SO_4$) (1:9 ratio by weight) catalyst. After digestion in an aluminum heating block, the concentration of NH_4-N was determined by automated colorimetry using the indophenol reaction. Organic C was determined by a modified Mebius method (hot chromic acid oxidation) as described by Soon and Abboud (1991). Soil pH was measured in a 0.01 M calcium chloride ($CaCl_2$) solution (1:2 soil–solution ratio). Nitrate in soil extracts was determined by an automated cadmium (Cd) reduction procedure and NH_4-N by the indophenol method.

The data were subjected to analysis of variance and correlation analysis. Nitrogen mineralized during aerobic and anaerobic incubation and NH_4-N extracted by hot KCl was expressed also as a percent of total N (i.e., normalized) to eliminate the effect of variations in total soil N on the correlation

between N mineralization indicators. A low significance probability associated with the F statistic ($P > F$) should indicate that the experimental variable is sensitive to the treatment effect.

RESULTS

Sensitivity of N Mineralization Indicators among 23 Soils

The average pH of the 23 soils was 5.42 (acid soil is typical of this region), and only one soil had a pH greater than 7.0. Other selected soil properties are presented in Table 1. The mean C:N ratio was 12. On average, hot KCl-hydrolyzable NH_4-N constituted slightly more than half of hot KCl-extractable NH_4-N (i.e., slightly less than half of extractable NH_4-N was exchangeable NH_4-N). Ammonia-N released by anaerobic incubation was greater than hot KCl-extractable NH_4-N. On average, 2.8% of TN was mineralized during the 24-day incubation at 25°C and 1.7% during the 7-day anaerobic incubation. Anaerobic mineralizable N and hot KCl-extractactable N were estimated with greater precision than aerobic mineralizable N and hot KCl-hydrolyzable N, whereas protease assay was the least precise (as indicated by their coefficients of variation). The relative range of values was highest for aerobic mineralizable N (48-fold difference between the maximum and minimum) and lowest was hot KCl-extractable N (6-fold difference between the maximum and the minimum). The hot KCl-extractable NH_4-N was the only mineralizable N indicator that displayed a narrower range than total N.

Table 1. Mean, standard deviation, and range in N-related properties of 23 soils

Properties	Mean	Std. dev.	CV (%)[a]	Range
Organic C (g kg^{-1})	24.2	14.3	nd	6.5–51.9
Total (Kjeldahl) N (g kg^{-1})	2.05	1.33	9.0	0.64–4.98
Mineralizable N (24-d incub.) (mg kg^{-1})	57.7	37.2	17.4	2.9–139
Mineralizable N (7-d anaer. incub.) (mg kg^{-1})	35.7	24.0	12.8	2.9–80.8
Hot 2 M KCl-hydrolysable NH_4-N (mg kg^{-1})	15.0	8.1	20.8	3.1–28.8
Hot 2 M KCl-extractable NH_4-N (mg kg^{-1})	24.9	12.8	12.7	7.5–45.4
Protease activity (nmol tyrosine g^{-1} h^{-1})	329	217	28.4	48.5–760

[a]CV: coefficient of variation.

Among the test indices, hot KCl-extractable NH_4-N and hot KCl-hydrolyzable NH_4-N were most strongly correlated with each other regardless of whether the values were normalized with respect to total N (Table 2). Aerobic mineralizable N and anaerobic mineralizable N were also strongly correlated with each other, but a little less so than were hot KCl-extractable NH_4-N and hot KCl-hydrolyzable NH_4-N. Correlations between the chemical indicators (e.g., hot KCl-extractable NH_4-N) and the biological indicators (mineralable N) were also highly significant. Somewhat surprisingly, the two chemical indicators (hot KCl-extractable and hydrolyzable NH_4-N) were more correlated with soil organic C and total N than were the biological indicators (aerobic and anaerobic mineralizable N). However, when the values were normalized, there was no correlation except for a negative one between total N and hot KCl-hydrolyzable NH_4-N. Protease activity was better correlated with the biological indicators than the chemical indicators, and there was no correlation when the chemical indicators were normalized with respect to total N. Protease activity was not correlated with total N, and the correlation with organic C was relatively weak.

Sensitivity to the Effect of Cropping Sequence

Crop rotation had no significant effect on any of the N mineralization indicators for the 0- to 15-cm depth of the 2000 Leith soil (Table 3). Results for hot KCl-hydrolyzable N (data not shown) were much more variable than for hot KCl-extractable N, probably because the former required correction for initial NH_4-N values and therefore, had an additional source of error.

Table 2. Correlation coefficients between N-mineralization indicators for 23 soils[a]

Indicator	Min-N	An-N	HA-N	HEA-N	TN	OC	Protease
Min-N		0.895***	0.794***	0.801***	0.644***	0.673***	0.799***
An-N	0.901***		0.865***	0.819***	0.668***	0.732***	0.773***
HA-N	0.626***	0.713***		0.963***	0.838***	0.850***	0.551**
HEA-N	0.573**	0.636***	0.942***		0.907***	0.918***	0.522**
TN	nc	nc	−0.452*	nc		0.964***	nc
OC	nc	nc	nc	nc	nd		0.475*
Protease	0.597**	0.579**	nc	nc	nd	nd	

[a]Values in italics in the lower triangle (i.e., below unfilled cells) are results obtained when N-mineralization indicator values are expressed as a percentage of soil total N. Abbreviations: Min-N: mineralizable N (24-d aerobic incubation); An-N: mineralizable N (7-d anaerobic incubation); HA-N: hot 2 M KCl-hydrolyzable NH_4-N; HEA-N: hot 2 M KCl-extractable NH_4-N; TN: total N; OC: organic carbon; nc: not correlated ($P > 0.05$); nd: not determined.

*, **, and *** indicate probability levels of 0.05, 0.01 and 0.001, respectively.

Table 3. Rotation and tillage effects on N released by aerobic and anaerobic incubation, hot KCl-extractable NH_4-N, and protease activity of Leith soil in 2000[a]

Treatment	Aerobic min-N (mg N kg^{-1})	Anaerobic min-N (0–5 cm) (mg N kg^{-1})	Anaerobic min-N (mg N kg^{-1})	Hot KCl-N (0–5 cm) (mg N kg^{-1})	Hot KCl-N (mg N kg^{-1})	Protease activity (nmol tyrosine h^{-1} g^{-1})
Crop sequence[b]						
P–W–C–W	18.9	33.6	17.8	82	11.1	71
Rc–W–C–W	22.6	48.1	21.3	76	11.9	74
F–W–C–W	24.1	34.1	18.2	70	11.0	60
SEM (20 DF)[c]	2.30	4.68	2.04	8.0	0.49	5.7
Pr > F[d]	0.27	0.07	0.44	0.59	0.34	0.23
Tillage						
Conventional	22.0	27.9	17.3	62	10.8	73
No till	21.7	49.4	20.9	90	11.9	63
SEM (2 DF)	1.68	1.46	1.38	4.9	0.42	3.6
Pr > F	0.91	0.01	0.32	0.06	0.19	0.18

[a]Values shown for each rotation are the means of the third and fourth courses of the rotations, and unless indicated otherwise are for soil samples to 15-cm depth. Mean for rotation effect is based on 12 observations and for tillage effect on 18 observations.

[b]Abbreviations for crop sequence: P, pea; W, wheat; C, canola; F, fallow; Rc, red clover.

[c]SEM: standard error of the mean; DF: degree of freedom.

[d]Pr > F: probability of a greater F value.

Initial NH_4-N concentrations were quite high and variable for this soil. The range of values of the biological and chemical indices of N mineralization was comparatively small for this data set. Confining measurements of N mineralization indices to the 0- to 5-cm depth improved the sensitivity of anaerobic mineralizable N to rotation effects compared to measurements of the 0- to 15-cm depth, although the F ratio was barely not significant ($P = 0.07$). However, there was no improvement for hot KCl-extractable and hydrolyzable NH_4-N. There was insufficient sample from the 0- to 5-cm depth for aerobic incubation.

Both biological and chemical indices of mineralizable N in the 0- to 15-cm depth of the 2002 Leith soil samples also failed to detect significant differences ($P \leq 0.05$) among crop sequences (Table 4). These samples had not been dried prior to the incubation, and initial NH_4-N was low. Consequently, hot KCl-hydrolyzable N values (data not shown) were only slightly lower than the equivalent extractable N. When samples from the 0- to 5-cm depth were used, mineralizable N concentrations were higher, and significant differences were found between the crop sequences for both aerobic and anaerobic mineralizable N. Although sensitivity of hot KCl-extractable N index was also increased, the P level improved to only 0.08. These indices consistently showed that mineralizable N was highest following red clover. The effect of the green manure was less evident two crops later (i.e., **Rc**–W–C–W vs. Rc–W–**C**–W). With the field pea rotation, there was no apparent difference in mineralizable N whether pea was grown previously or two crops before.

Sensitivity to the Effect of Tillage Practices

The biological and chemical indicators of mineralizable N in the surface 15 cm of the 2000 Leith soil showed no significant response to tillage effects (Table 3). However, when only the top 5 cm of soil was used, anaerobic mineralizable N showed a significant effect of tillage, while the probability of the null hypothesis for hot KCl-extractable N improved to 0.06. Whereas both aerobic and anaerobic mineralizable N and hot KCl-extractable N were not significantly different as a result of tillage for the top 15 cm of the 2002 Leith soil, they were all greater under NT than CT when measured using only the top 5 cm of soil, with the $Pr > F$ value improving to 0.10 for anaerobic mineralizable N, 0.05 for hot KCl extractable N, and 0.01 for aerobic mineralizable N (Table 4).

Aerobic mineralizable N and hot KCl-extractable N showed a significant difference due to tillage effects in the Hythe soil (0–10 cm), whereas anaerobic mineralizable N failed to detect any significant difference (Table 5). Values for hot KCl-hydrolyzable N (data not shown) were also significantly different between tillage treatments and were only slightly lower than those for hot KCl-extractable N because of low initial concentrations of NH_4-N.

Table 4. Effect of crop sequence and tillage on three N mineralization indicators of Leith soil to two depths in 2002[a]

Treatment	Aerobic incubation		Anaerobic incubation		Hot KCl-extracted N	
	0–5 cm (mg N kg^{-1})	0–15 cm (mg N kg^{-1})	0–5 cm (mg N kg^{-1})	0–15 cm (mg N kg^{-1})	0–5 cm (mg N kg^{-1})	0–15 cm (mg N kg^{-1})
Crop sequence[b]						
Rc–W–**C**–W	52.5	19.1	78.5	50.3	14.7	11.6
P–W–C–W	45.6	16.4	68.9	59.3	14.0	12.4
F–W–C–W	39.0	11.9	59.8	45.7	12.7	10.0
Rc–W–C–W	58.1	25.1	114.1	60.7	18.1	12.0
P–W–C–W	47.2	17.8	67.1	50.8	12.3	10.4
Cont. W	52.5	24.4	71.4	49.4	16.5	12.2
SEM (19 DF)[c]	3.83	3.11	7.28	4.84	1.38	0.75
Pr > F[d]	0.04	0.10	0.001	0.21	0.08	0.21
Tillage						
Conventional	42.5	19.2	58.4	50.6	11.6	10.3
No till	55.2	18.7	94.1	54.8	17.6	12.4
SEM (2 DF)	0.37	4.14	8.52	4.69	0.97	0.76
Pr > F	0.01	0.97	0.1	0.54	0.05	0.17

[a]Soils sampled in spring 2002 and not dried prior to analysis. Number of observations per mean is 6 for crop sequence effect and 18 for tillage effects.

[b]Bold letters indicate previous crop. P is abbreviation for pea, W for wheat, C for canola, F for fallow, and Rc for red clover.

[c]SEM: standard error of the mean; DF: degree of freedom.

[d]Pr > F: probability of a greater F value.

Table 5. Effect of tillage and lime on three N mineralization indicators of Hythe soil (0–10 cm)[a]

Treatment	Aerobic incubation (mg N kg^{-1} soil)	Anaerobic incubation (mg N kg^{-1} soil)	Hot KCl extracted-N (mg N kg^{-1} soil)
Lime (Mg ha^{-1})			
0	48.8	70.1	25.2
7.5	61.4	90.3	24.8
Pr > F[b]	0.01	0.01	0.68
Tillage			
Conventional	50.8	77.3	22.9
No tillage	59.5	83.1	26.9
Pr > F	0.04	0.38	0.01
SEM (39 DF)[c]	2.92	4.64	1.09

[a]Number of samples per mean = 24.
[b]Pr > F: probability of a greater F value.
[c]SEM: standard error of the mean; DF: degree of freedom.

Sensitivity to the Effect of Liming

Only the biological N mineralization indicators, aerobic and anaerobic mineralizable N, showed a significant difference due to soil liming (Table 5). Liming induced even bigger differences than did tillage in the amount of N mineralized. However, hot KCl-extractable N did not detect any difference. Values of hot KCl-hydrolyzable N (data not shown) were essentially similar to those of hot KCl-extractable N because of very low initial values of KCl-extractable NH_4-N of this soil. Protease activity measurements were not done on this soil to test its sensitivity to liming.

DISCUSSION AND CONCLUSIONS

The ranges and means of anaerobic mineralizable N, hot KCl-extractable and hydrolyzable N, and protease activity of the 23 farm soils are of similar order to those previously reported (Keeney and Bremner 1966; Gianello and Bremner 1986b; Jalil et al. 1996; Ladd and Butler 1972; Ross and McNeilly 1975). Jalil et al. (1996) considered it desirable for an N-availability index to have a wide range in values. In addition, a test should be simple to perform, rapid, and precise. All soil N indicators tested for the 23 soils showed significant correlations with each other and were sensitive enough in showing significant differences among soils. Ammonia-N released during anaerobic incubation had the strongest correlation with mineralizable N

(obtained by aerobic incubation), followed by hot KCl-hydrolyzable NH_4-N. The high correlation reflects a probable common origin for at least a portion of their N. The microbial biomass N is a major source of N mineralized during anaerobic incubation of soil (Myrold 1987; Stockdale and Rees 1994). This should not be surprising because mineralization involves mainly microbiologically mediated processes. The protease assay was the least precise of the methods tested, probably because of the small sample size used, and it assessed the potential of only a small part of the entire mineralization process, the cleavage of amino acids from protein molecules. The relatively high variability and complexity of protease activity measurement and its laborious nature make its adoption as an N mineralization indicator unlikely. The anaerobic incubation and hot KCl-extraction procedures possess most of the desired attributes of an N mineralization indicator, although the range of the latter for the 23 soils was not as wide as that of some other indicators. The chemical indicators were not correlated with microbial biomass N or plant-available N (Stockdale and Rees 1994; Xu et al. 1996; Wang et al. 2001). An issue with the hot KCl procedure is whether to subtract the initial NH_4-N content. This study showed that when initial NH_4-N values are high and variable, a more sensitive test resulted if no correction was applied. Therefore, in concurrence with Jalil et al. (1996), it is concluded that hot KCl-extractable N should be preferred over hot KCl-hydrolyzable N as a chemical test of N mineralizability. Jalil et al. (1996) had previously reported that the test was not different if no correction for initial NH_4-N was made, thereby making the method yet simpler.

Sensitivity of a test to management effects within a soil type or association should be a critical criterion for its adoption for measuring soil-quality attributes. Sensitivity of the tests to soil and crop management effects improved when measurements were confined to shallower depths of soil (e.g., 5 cm). This was shown for tillage as well as crop sequence effect by this study. In NT system, crop residues were not incorporated and, therefore, stayed at the soil surface. Consequently, greater mineralization occurred within the top 5 cm of soil because of higher moisture and organic matter content as well as greater microbial activity (Doran 1987). El-Haris et al. (1983) found that soils displayed greater differences in mineralizable N when sampled in spring than in autumn. The results of this study tend to agree, although the difference here could also be attributed to using soils kept moist vs. air dried prior to the test (Tables 3 and 4). Generally, aerobic incubation tended to be a more sensitive test than anaerobic incubation. If the 24-d aerobic incubation procedure is deemed too laborious and time-consuming, an experimenter has probably two choices: the anaerobic incubation or a chemical method (e.g., hot KCl extraction). The anaerobic mineralizable N was significantly different ($P \leq 0.05$) between NT and CT in one of three tests for soils taken from no deeper than 10 cm from field experiments, and the hot KCl procedure (the hot KCl extractable N, in particular) in two of three tests. Liebig, Tanaka, and Wienhold (2004) also found that NT increased

anaerobic mineralizable N compared to CT. Although anaerobic mineralizable N was somewhat less sensitive than hot KCl-extractable N to tillage effects, it was also significantly different among crop sequences in one of two tests, and hot KCl-extractable N was not. Here the lack of a relationship between chemical procedures and biologically labile N may have been a factor in the chemical indices' insensitivity to a crop sequence effect.

The anaerobic N procedure also found a significant effect of liming on mineralizable N (as did the aerobic incubation) but the hot KCl tests did not. Nyborg and Hoyt (1978) reported that liming doubled the amount of N mineralized from acid soils. Liming has been shown to result in higher microbial biomass N accumulation (Soon and Arshad 2005). It is probably the turnover of the increased microbial N that resulted in higher mineralizable N associated with liming of acid soils. The tendency for soil mineralizable N and crop N uptake to be higher following a legume is well documented (Francis, Haynes, and Williams 1991). Soon, Clayton, and Rice (2001) attributed the greater N availability following pea or red clover than continuous wheat to higher turnover of microbial biomass N and/or crop residue N. The crop sequence effect was most apparent following a legume green manure. El-Haris et al. (1983) suggested that increased organic N and C input from the incorporation of green manure compared to other rotations is reflected in enhanced N mineralization. Because the decomposition of crop residues involves a suite of soil microorganisms, the superiority of the biological indicators over the chemical indicators is not so surprising.

It is concluded that waterlogged incubation provided a satisfactory test of management effects on soil mineralizable N and is a suitable compromise between the longer and laborious aerobic incubation procedure and the more rapid hot KCl procedures.

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