Soil Nitrogen Mineralization Influenced by Crop Rotation and Nitrogen Fertilization

Lynne Carpenter-Boggs,* Joseph L. Pikul Jr., Merle F. Vigil, and Walter E. Riedell

ABSTRACT

An estimate of soil mineralizable N is needed to determine crop needs for N fertilizer. The objective of this research was to estimate soil net N mineralization in soils maintained in continuous corn (*Zea mays* L.) (CC), corn–soybean [*Glycine max* (L.) Merr.] (CS), and corn–soybean–wheat (*Triticum aestivum* L.)/alfalfa (*Medicago sativa* L.)–alfalfa (CSWA) rotations that have been managed since 1990 with zero N (0N), low N (LN), and high N (HN) fertilization. Soil samples were taken from 0- to 20-cm depth in plots planted to corn in 1998. In order to produce more realistic time-series data of net N mineralization, soils were incubated in filtration units in a variable-temperature incubator (VTI) that mimicked field soil temperatures under a growing corn canopy. Rotation and N fertilization significantly affected net N mineralization in soil samples. Cumulative net N mineralized in a 189-d field temperature incubation averaged 133 ± 6 kg ha^{-1} in CC, 142 ± 5 kg ha^{-1} in CS, and 189 ± 5 kg ha^{-1} in CSWA. Across rotations, average net N mineralized was 166 ± 9 kg ha^{-1} in 0N plots, 147 ± 10 kg ha^{-1} in LN plots, and 152 ± 10 kg ha^{-1} in HN plots. Inclusion of a legume, particularly alfalfa, in the rotation increased net N mineralized. Generally, more net N was mineralized from plots receiving no fertilizer N than from soil with a history of N fertilization. Variable-temperature incubation produced realistic time-series data with low sample variability.

Given favorable growth conditions, N availability is one of the most variable and critical factors in determining crop yield. Net N mineralization from organic sources in soil is estimated using knowledge of local soils and climate in order to predict realistic yield goals, N fertilization requirements, and inherent soil fertility. Fertilizer N recommendations generally are based upon the crop yield goal, NO_3 available in the soil at planting, and N credits for any legume grown or manure applied the previous year. All N sources are considered additive, and fertilizer recommendations have not addressed possible interactions between organic-derived N and commercial N fertilization. Commercial fertilization can increase crop growth, decrease crop residue C/N ratio by increasing stover N concentration (Liang and Mackenzie, 1994), reduce N fixation by legumes (Streeter, 1988), and increase (Entry et al., 1996; Conti et al., 1997) or decrease (Biederbeck et al., 1984; Ladd et al., 1994; Smolander et al., 1994) soil microbial populations and activities. Because commercial N fertilization can affect N fixation, mineralization of residue or soil organic N, and other biological processes, commercial fertilization could affect the level of N made available to crops that follow legumes in rotation.

While fertilization guides use total organic matter and previous crop as indicators of N mineralization for the coming season, a variety of direct and indirect lab methods may be used for more precise predictions (Fox and Piekielek, 1978; Hong et al., 1990). Laboratory tests allow compositing and homogenizing soil samples to decrease the standard deviation and required replication. Aerobic incubation for 120 to 252 d is commonly used to estimate the size and decay rates of mineralizable N pools (Stanford and Smith, 1972; Cabrera and Kissel, 1988). Temperature and matric potential of incubated soils affect the rate and cumulative N mineralized. Within ordinary field soil matric potentials from −1.85 to −0.01 MPa, temperature has a greater influence on N mineralization than does matric potential (Zak et al., 1999). Most N mineralization laboratory experiments are incubated at 35°C, considered the ideal temperature for maximum N mineralization. Nitrogen mineralized in laboratory incubations at 35°C represents potential N mineralization and first-order rate constants, not field N mineralization as affected by field soil conditions.

In situ N mineralization can be determined using in-field undisturbed soil cores, usually in conjunction with ion exchange resins (DiStefano and Gholz, 1986). In situ cores are exposed to field temperature and sometimes field moisture, whereas lab tests generally involve incubation under ideal temperature and moisture conditions. In addition, intact cores are not subject to physical disturbance, which can expose protected organic materials and increase the estimate of labile N. However, field methods are subject to large standard deviations because they use individual soil cores rather than composite samples. This large variability between replicates necessitates use of large numbers of replicates for each treatment or plot. In situ measurement of N mineralization at two sites in eastern Colorado required five to seven soil core replicates per plot to obtain a small enough variance to detect a 3.0 kg N ha^{-1} difference at an α level of 0.20 (Kolberg et al., 1997). Comparison of several replicated field treatments with adequate replication of periodically replaced in situ cores quickly becomes a daunting task.

The primary objective of this study was to determine the effects of three rotations at three N fertilization levels on net N mineralization in an eastern South Dakota soil. A second objective was to evaluate a laboratory method for measurement of net N mineralization that more accurately reflected the effects of field temperatures rather than constant optimal incubation temperature.

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Abbreviations: 0N, zero N fertilizer; CC, continuous corn; CS, corn–soybean; CSWA, corn–soybean–wheat/alfalfa–alfalfa; HN, high N fertilizer; LN, low N fertilizer; VTI, variable-temperature incubator.

MATERIALS AND METHODS

Study Site

Study plots are located on the Eastern South Dakota Soil and Water Research Farm at Brookings, SD. Rotation and N application rates have been consistent since spring 1990. The soil is Barnes clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludoll) with nearly level topography. Average site conditions are described in Table 1. The experimental design includes three replicate blocks of three crop rotations: CC, CS, and CSWA. All crops in each rotation are grown each year. Each 91 by 30 m rotation plot was split into three randomized 30 by 30 m subplots to test fertilization effects at 0N, LN, and HN fertilization. Nitrogen application for corn in 0N, LN, and HN treatments was based on yield goals of 0, 5.3, and 8.5 Mg ha^{-1}, respectively. For these yield goals, total available N required for 0N, LN, and HN was 0, 114, and 181 kg N ha^{-1}, respectively. Preplant soil NO_3 in the top 120 cm was subtracted from N requirement to determine fertilizer needs. Starter fertilizer was applied to all plots in corn, soybean, and wheat phases of rotations. Starter fertilizer contained 18 kg ha^{-1} P, 12 kg ha^{-1} K, and 0, 8, or 16 kg ha^{-1} N in 0N, LN, and HN plots, respectively. After subtracting preplant soil NO_3 and starter fertilizer from total N needs, the remaining N requirement in corn was applied as sidedressed urea. Soybean and wheat received only starter fertilizer as described above; alfalfa received no fertilizer.

In CSWA, alfalfa was companion seeded along with wheat in the spring, but attained minimal growth until wheat was harvested. Alfalfa was not harvested during the wheat year, but was harvested as hay normally three times in the fourth year of CSWA rotation. After the final cutting alfalfa was allowed 3 to 4 wk regrowth, then killed with glyphosate [*N*-(phosphonomethyl)glycine; Roundup, Monsanto, St. Louis, MO] ~6 wk before fall chisel tillage. Corn and soybean grains were harvested and removed from plots and corn stover was chopped and remained on plots.

Through spring 1996, primary tillage was by moldboard plow (0–20 cm) in the fall, weather permitting, or in spring otherwise. After spring 1996 moldboard plowing was discontinued; since that time plots were chisel plowed (0–15 cm) in the fall, then prepared each spring using a tandem disk and field cultivator (0–5 cm). All corn and soybean plots were row-cultivated twice each year. Seeding date, rate, and variety were the same for all plots in a given year.

Crop Measurements

Samples to determine biomass and N uptake were harvested by hand when the crop was mature, just prior to field grain harvest. Soybean biomass samples were taken just before leaf drop. All plant material was cut from four rows 1 m long. Bundles were dried 3 d at 50°C with circulating air and then weighed. Total aboveground biomass was separated into grain and stover components. Grain and stover were thoroughly ground to pass a 0.5-mm sieve and analyzed for C and N content using a Carlo-Erba elemental analyzer (CE Instruments, Milan, Italy). Weighted residue C/N ratio was determined by C/N ratio and proportion of total biomass produced by each crop in rotation.

Nitrogen Mineralization and Other Laboratory Methods

On 28 Apr. 1998, 2 d prior to corn seeding, fourteen 3.4-cm-diam. cores were taken in the top 20 cm of each plot that would be seeded to corn. Previous crop was corn in CC, soybean in CS, and alfalfa in CSWA. Entire composite samples consisting of 14 soil cores were weighed and a sample dried at 105°C to determine initial bulk density and moisture. Soils were passed through a 4-mm sieve and material larger than 4 mm was removed.

Table 1. Average climate in 1990 through 1998 and soil conditions in 1998 at soil depth of 0 to 20 cm at study site near Brookings, SD.

Parameter	Value†
Annual precipitation, mm	620
Annual mean temperature, °C	5.8
Annual growing degree days (base 10°C)	1 320
Soil texture	Clay loam
Bulk density, g cm^{-3}	1.14 (0.15)
Moisture at sampling, g kg^{-1}	176 (18)
Electrical conductivity, S m^{-1}‡	0.03 (0.006)
pH§	6.6 (0.5)
Soil C/N ratio	10.9 (0.2)
Organic C, mg kg^{-1}¶	18 300 (1700)
Total N, mg kg^{-1}¶	1 680 (160)
Soluble C, mg kg^{-1}#	27 (8)
P, mg kg^{-1}††	19 (5)
K, mg kg^{-1}‡‡	160 (10)

† Values are means (n = 27) with standard deviation in parentheses.
‡ D.A. Whitney, 1998.
§ 1:1 soil/0.01 mol L^{-1} $CaCl_2$ (Watson and Brown, 1998).
¶ Leco CN-2000 (Leco, Nelson and Sommers, 1996).
# Accu-Test Chemical Oxygen Demand digest (Bioscience, Inc., Bethlehem, PA).
†† Olsen P test (Frank et al., 1998).
‡‡ Warncke and Brown, 1998.

Net N mineralization, or the difference between organic N decomposition and inorganic N immobilization, was tracked throughout the growing season using a modification of the procedure of Stanford and Smith (1972). In order to minimize replicate variability while increasing relevance of laboratory data to field conditions, net N mineralization was measured in the laboratory during incubation at field soil-mimicking temperatures. Disposable 150-mL Falcon filtration units (Corning Costar, Corning, NY) were prepared within 1 d of sampling. Units had 0.22-μm-pore cellulose acetate filters and glass fiber prefilters. Two filtration units per plot were filled with the field-moist equivalent of 30 g dry weight soil mixed with 30 g washed silica sand. Additional 75-mm glass fiber prefilters (Millipore Corporation, Bedford, MA) were placed above soil–sand mixture to inhibit soil dispersion during addition of leaching solution. Units were leached on Days 0, 14, 28, 42, 63, 85, 106, 126, 146, 167, and 189 with 100 mL of 0.025 mol L^{-1} $CaCl_2$. Units were evacuated at −80 kPa until 90 to 95 mL of solution was retrieved. Solution was filtered through Whatman no. 42 filters and frozen for later analysis of NO_3 and NH_4. Twenty milliliters of nutrient solution containing 0.005 mol L^{-1} $MgSO_4$ and 0.005 mol L^{-1} KH_2PO_4, brought to pH 7.2 with KOH, was then added to filtration units and vacuum applied at −80 kPa for 30 min. Moisture content of the soil–sand mixture averaged 220 g kg^{-1} prior to leaching with $CaCl_2$ solution and 260 g kg^{-1} just after 30 min vacuum. Between leachings, units were sealed with Parafilm (American National Can Co., Greenwich, CT) and incubated in a VTI that mimicked the temperature in topsoil under a crop canopy (Fig. 1a). A corn plot was planted near the laboratory on the date of field planting. A thermistor placed 10 cm deep in the soil between rows in this corn plot was connected to the VTI temperature controls inside the laboratory.

Two additional sets of filtration units were prepared with soil from all plots under CS LN management. One set was kept in an incubator at 17°C and one set at 35°C for comparison of temperature effects on net N mineralization. Other than

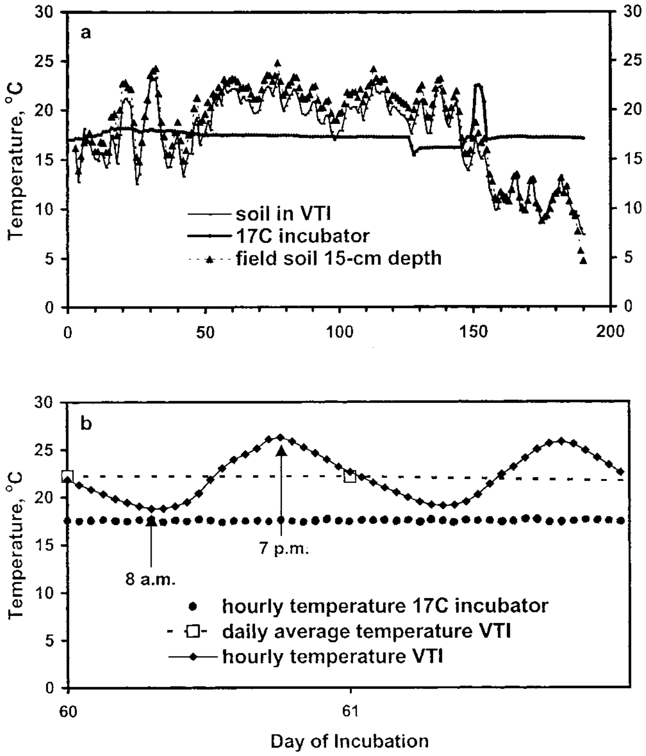

Fig. 1. (a) Daily mean temperature during incubation period in field soil at 15-cm depth under growing corn canopy compared with soil in filtration units in 17°C incubator and variable temperature incubator (VTI) mimicking field soil temperatures. (b) Detail of hourly temperature in VTI and 17°C incubators during incubation, Days 60 and 61.

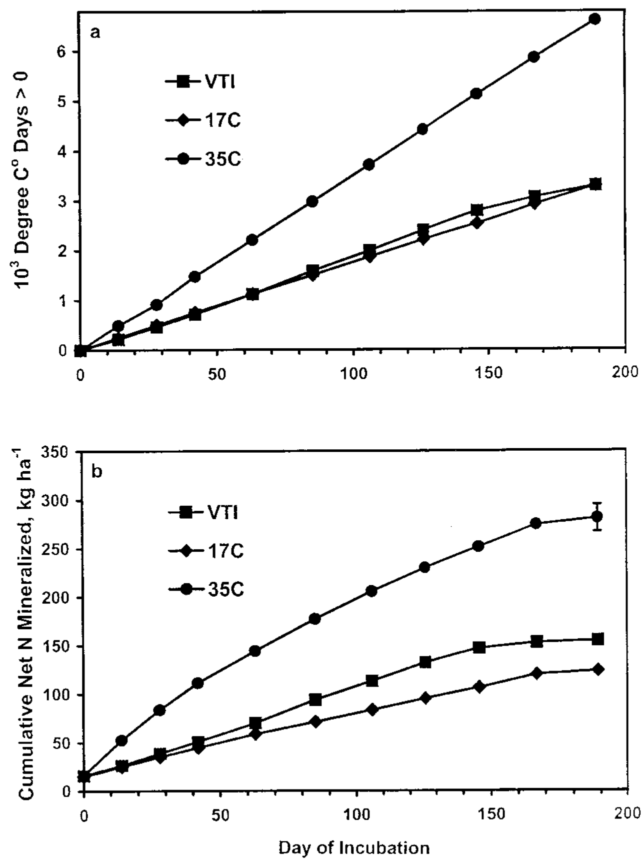

Fig. 2. (a) Accumulation of degree Celsius days >0°C and (b) mineralized N in soil taken from 0 to 20 cm in corn–soybean rotation with history of low N fertilization of 114 kg N ha^{-1}. Subsamples were incubated at constant temperature of 35 or 17°C, or in the variable temperature incubator (VTI) mimicking field soil temperature at 15-cm depth under growing corn canopy. Bars on final data points of mineralized N indicate ± one standard error of the mean. Error bars not visible on VTI and 17°C because error bars are smaller than data points.

temperature, the treatment and leaching of these units was identical to that of filtration units in the VTI.

Nitrate and NH_4 in leachates were analyzed on a Lachat Instruments (Milwaukee, WI) autoanalyzer (Zellweger Analytics, 1992). Results were converted to kilograms N per hectare plow layer using field bulk density measurements in the top 20 cm of each plot. Nitrate and NH_4 were summed at each leaching to express initial inorganic N (Day 0) or net N mineralized. Initial inorganic N plus cumulative net N mineralized represent total available N.

Statistical Analysis

Replicate measurements on composite soil samples were averaged for statistical analysis of treatment effects. Analysis of main effects and interactions was completed using General Linear Models (GLM) in SYSTAT 9.0 (SPSS Inc., Chicago, IL). Because of the split-plot design, hypotheses of rotation effects were tested using whole plot effects (rotation × block) as error term. Significant differences were determined using GLM factor effects and least significant differences at $P \leq 0.05$. Results are reported as mean and standard deviation of the mean unless otherwise noted.

RESULTS AND DISCUSSION

Field Temperature-Mimicking Incubation

Temperature of soils in the VTI chamber closely mimicked temperature in the soil outdoors at 15-cm depth under a growing corn canopy (Fig. 1a). During the 189-d incubation period, soil temperatures at 15-cm depth in the field averaged 18.5°C and both the 17°C incubator and VTI averaged 17.4°C. Although the thermistor was placed in the field soil at a 10-cm depth, lag time in changing temperatures of incubator air and soil caused the VTI soil temperature cycles to more closely approximate temperatures at a 15-cm depth in the field. Daily mean temperatures (Fig. 1a) mask the higher temperatures in the VTI during afternoon and evening hours (Fig. 1b), which probably stimulated greater mineralization during these periods. Cumulative degree days in the 17°C incubator and VTI diverged during incubation Days 60 to 140 (Fig. 2a), when field soil and variable-temperature incubator temperatures exceeded 17°C (Fig. 1a). Net mineralization of N was also greater in the VTI than in the 17°C incubator during this period (Fig. 2b). Although temperatures were subsequently greater in the 17°C incubator than the VTI on Days 146 to 189 (Fig. 1a) and cumulative degree days were equal by Days 189 (Fig. 2a), cumulative N mineralization in 17°C incubator did not match that in the VTI (Fig. 2b). The rate constant (k) for N mineralization conforms to a Q_{10} of ~2 (Stanford et al., 1973), so that a temporary increase in temperature can cause a greater than propor-

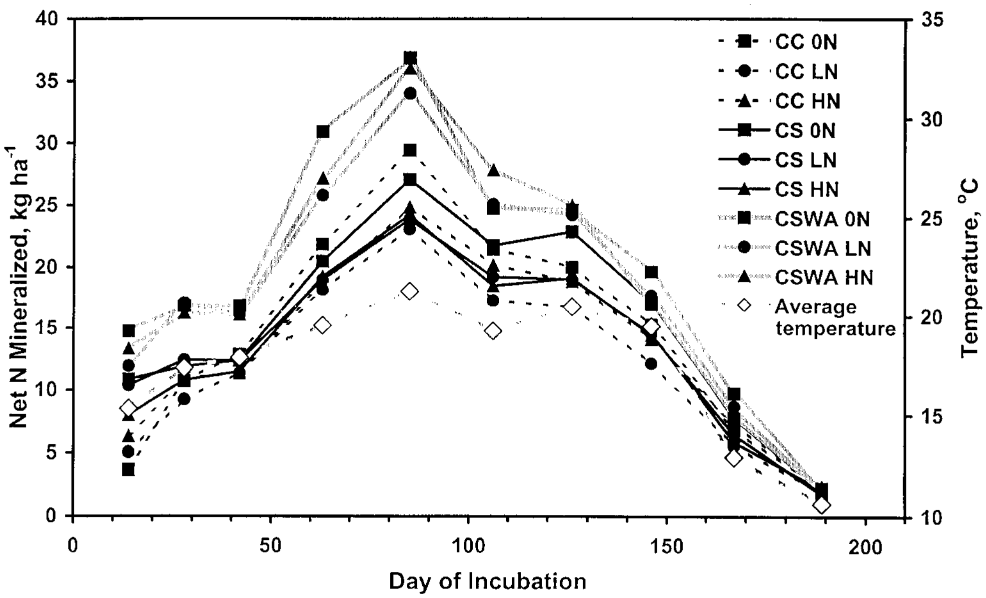

Fig. 3. Nitrogen leached from filtration units after each of 11 incubation periods and average daily temperature during each incubation period. Net mineralized N data are the mean of duplicate soil samples from each of three field replicates of continuous corn (CC), corn–soybean (CS), and corn–soybean–wheat/alfalfa–alfalfa (CSWA) rotations managed at zero N (0N), low N (LN), or high N (HN) fertilization incubated at field soil temperature.

tional increase in the rate of N mineralization. Daily temperature fluctuations above and below the daily mean temperature in the VTI can thereby stimulate more mineralization than a constant-temperature incubator at the same daily average temperature.

The temporal pattern of net N mineralization produced by leaching units in the VTI reflects net mineralization dynamics as affected by temperature fluctuations and initial soil disturbance (Fig. 3). Unlike most laboratory incubations at a constant temperature of 35 to 40°C, soils in the VTI did not produce a large flush of mineralized N in the first few weeks. Temperature in the VTI averaged 17°C during the first three incubation periods (total 6 wk). Although stimulation of microbial activity due to sample disturbance is inevitable, a moderate initial incubation temperature and lack of grinding, drying, and rewetting of soil samples minimized the initial flush of activity and N release.

Initial and Net Mineralized Nitrogen

Initial inorganic N was significantly affected by rotation but not by fertilization (Table 2, Fig. 4). Soils had more inorganic N in the spring after alfalfa (in CSWA) than after soybean (in CS), which had more than after corn (in CC). Approximately 95% of initial inorganic N was present as NO_3 (data not shown). Spring preplant soil NO_3 is often considered residual N from the previous year, but in this study previous fertilization did not affect initial levels of inorganic N in spring soil samples. Preplant surface soil NO_3 has previously been well correlated with soil N supplying capability (r = 0.75, Hong et al., 1990). Initial inorganic N was well correlated with N mineralized in the 189-d VTI incubation when all rotations were considered (r = 0.88) (Fig. 5). However, initial inorganic N was only weakly correlated with mineralized N within any individual rotation. This suggests that rotation was an important factor in determining both initial inorganic N and N mineralized in these soils.

Table 2. Significance ($P < F$) of field treatments affecting initial spring inorganic N (NO_3 + NH_4) and N mineralization in soils sampled at the 0- to 20-cm depth before corn planting in 1998.

Effect	Initial inorganic N	N mineralized 189 d	Total available N
		$P < F$	
Block	0.05	0.61 (NS)†	0.37 (NS)
Rotation	0.001	0.02	0.01
Fertilization	0.76 (NS)	0.01	0.02
Fertilization × rotation	0.17 (NS)	0.62 (NS)	0.45 (NS)

† NS is not significant ($P > 0.05$).

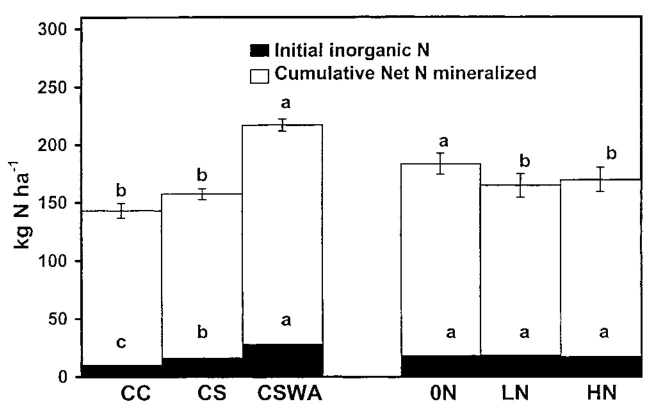

Fig. 4. Initial inorganic N (NO_3 + NH_4) plus cumulative mineralized N extracted in repeated leachings during a 189-d incubation from 29 April to 4 Nov. 1998. Data are means of two duplicate soil samples from three replicates of continuous corn (CC), corn–soybean (CS), and corn–soybean–wheat/alfalfa–alfalfa (CSWA) rotations managed at zero N (0N), low N (LN), or high N (HN) fertilization incubated at field soil temperature. Error bars represent ± one standard error of the mean. For each measurement, bars within fertilizer or rotation treatment with the same letter are not significantly different according to Tukey's honestly significant difference ($P < 0.05$).

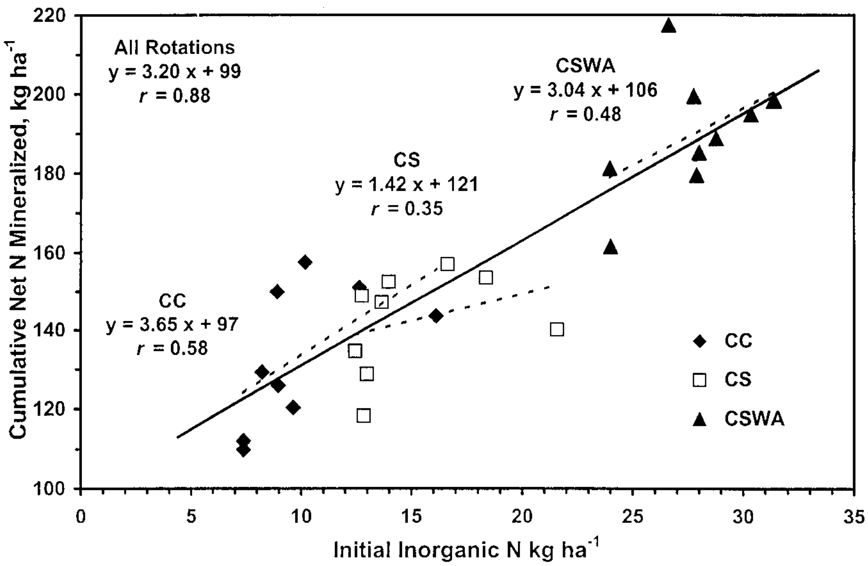

Fig. 5. Cumulative N mineralized, excluding initial inorganic N, vs. initial inorganic N (NO_3 + NH_4) in soil samples from continuous corn (CC), corn–soybean (CS), and corn–soybean–wheat/alfalfa–alfalfa (CSWA) rotations incubated 189 d at field soil temperature.

Linear models using initial inorganic N data to predict N mineralized in 189 d set the *y* intercept at ≈100 kg N ha^{-1} (Fig. 5). This suggests that a similar soil with no inorganic N at planting may mineralize ≈100 kg N ha^{-1} during 189 d under similar conditions.

Cumulative N mineralized from soils during the 189-d incubation in the VTI was significantly affected by both previous crop and previous fertilization level (Table 2, Fig. 4). Approximately 95% of mineralized N was present as NO_3 at each leaching (data not shown). Coefficient of variation in duplicate filtration units averaged 4.4%, compared with 13.5% in five replicates used in the in situ study of Kolberg et al. (1999).

Incubated soil samples represent only the upper 20 cm of field soil. Incubation temperatures in the VTI mimic those at 15-cm depth in soil under a growing corn canopy, and moisture generally ranged from 220 to 260 g kg^{-1}. Thus, these measurements can only represent a portion of the environmental complexity and N mineralization in the full soil profile, but probably correlate with field mineralization.

The relevance of these laboratory measurements to N mineralization in the field is supported by correlation of corn N uptake in the field to N mineralized in the VTI. Total N uptake by aboveground corn biomass in unfertilized plots in all rotations correlated well with total inorganic N from samples in the VTI (r = 0.89, Fig. 6). Alternatively, if corn N use efficiency in these plots was 45.45 kg corn kg^{-1} N (Gerwing and Gelderman, 1996), the average corn yield of 5.7 Mg in unfertilized plots would have required ≈126 kg ha^{-1} total available N. During the period of N uptake from planting to R-5 (dent; Ritchie et al., 1992) reached on Day 120, N mineralization in the VTI plus initial inorganic N averaged 146 kg ha^{-1}, overestimating predicted field N availability by only 20 kg ha^{-1}, or 16%.

The benefit of legumes to soil N supply is well established and has been observed for generations (Piper and Pieters, 1922). In the corn year of all rotations, soils managed in CSWA, CS, and CC mineralized the equivalent of 189, 142, and 133 kg N ha^{-1}, respectively. In other terms, soil under CSWA rotation mineralized 33 and 42% more N than soil under CS or CC rotations, respectively (Fig. 4). Dou et al. (1996) measured only 19% more N mineralization in soils after alfalfa than after fertilized or unfertilized continuous corn. Total available N (initial inorganic N plus N mineralized during 189 d) was 217, 157, and 143 kg N ha^{-1} in CSWA, CS, and CC, respectively.

Nitrogen fixation by *Rhizobium* spp. bacteria in symbiosis with alfalfa produces plant material with a relatively small C/N ratio. The previous crop of alfalfa harvested from CSWA plots in 1997 had a C/N ratio of 14:1, while corn residue in CC plots had a C/N ratio of 67 to 111:1, and soybean from CS plots had a C/N ratio of 36 to 55:1, depending on fertilization (Table 3). The return of alfalfa shoot and root residue to the soil supplies readily decomposable organic matter and readily mineralizable N. Aboveground biomass of alfalfa aver-

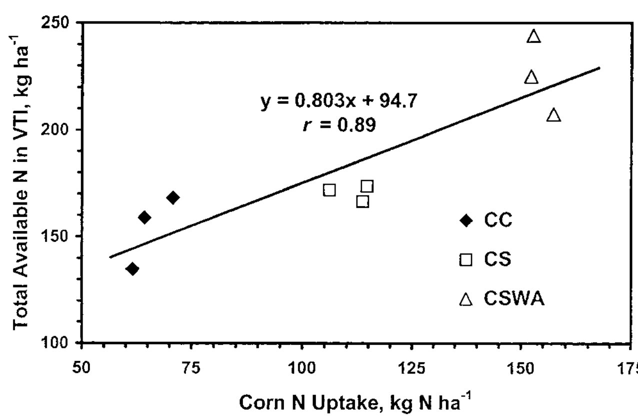

Fig. 6. Total available N (cumulative net N mineralized plus initial inorganic N) from soils incubated at field soil temperature vs. total aboveground biomass uptake of N in corn grown in unfertilized continuous corn (CC), corn–soybean (CS), and corn–soybean–wheat/alfalfa–alfalfa (CSWA) rotations.

Table 3. Yield and C and N content of crops grown in 1997 prior to corn in continuous corn (CC), corn–soybean (CS), corn–soybean–wheat/alfalfa–alfalfa (CSWA) rotations.†

Rotation	1997 Crop	Fertilizer N level‡	Stover or hay mass§	Stover or hay C	Stover or hay N	Stover or hay C/N
			kg ha^{-1}			
CC	Corn	0N	4700 (520)	2040 (240)	18 (2.7)	111 (3.7)
		LN	6430 (520)	2820 (260)	34 (6.6)	82 (9.1)
		HN	7630 (460)	3380 (220)	51 (11)	67 (12)
CS	Soybean	0N	2110 (390)	828 (170)	23 (18)	36 (21)
		LN	2080 (150)	803 (66)	20 (4.9)	40 (15)
		HN	2130 (480)	815 (200)	22 (9.6)	39 (9.5)
CSWA	Alfalfa	0N	8740 (250)	3890 (94)	286 (14)	14 (0.3)
		LN	9260 (770)	4100 (340)	303 (34)	14 (0.4)
		HN	8790 (550)	3910 (240)	285 (22)	14 (0.3)

† Values are means ($n = 3$) with standard deviation in parentheses.
‡ 0N, zero N fertilizer; LN, low N; HN, high N. Treatments 0N, LN, and HN fertilized for corn yield goal of 0, 5.3, and 8.5 Mg ha^{-1}, respectively.
§ Stover mass of corn and soybean is total aboveground biomass production excluding grain. Hay mass of alfalfa is sum of three cuttings during growing season.

aged 291 kg N ha^{-1} across fertility treatments, while biomass of corn and soybean residues averaged 34 and 21 kg N ha^{-1}, respectively. However, all non-grain biomass of corn and soybean was returned to soil with tillage, whereas most aboveground biomass of alfalfa was harvested as forage. The actual amount of alfalfa C and N returned to soil is unknown, but probably consisted primarily of root growth and exudates. Most legume-derived N benefitting subsequent crops is released in the first year after legume plow-down (Varco et al., 1993; Vanotti and Bundy, 1995). Averaged across fertilizer treatments, alfalfa supplied 60 and 74 kg ha^{-1} more available N than previous crops of soybean or corn, respectively.

Across rotations, 0N soils that had not received commercial N fertilizer for the previous 8 yr mineralized more net N than their fertilized counterparts (Fig. 4). Soils under 0N management mineralized on average 166 kg N ha^{-1}, while LN and HN treatments mineralized significantly less at 147 and 152 kg N ha^{-1}, respectively. This occurred despite a general trend toward greater average annual biomass yield, more biomass N, and lesser average C/N ratio of biomass in HN plots in every rotation (Table 4). Several previous studies have found a positive correlation between crop fertilization and soil N mineralization (Bonde and Rosswall, 1987; Gill et al., 1995; Kolberg et al., 1999). Others have noted no effect of fertilization on N mineralization (Franzluebbers et al., 1994). Negative fertilizer–mineralization interaction has been documented previously (McAndrew and Malhi, 1992; Wienhold and Halvorson, 1999), but less commonly than positive interactions.

The negative relationship of net N mineralization and N fertilization suggest that N fertilization may decrease net N mineralized or 0N management may increase net N mineralized in these soils. Decreased net N mineralization can result from less gross N mineralization or increased N immobilization. If decreased net mineralization in N-fertilized soils was caused by decreased gross N mineralization, some possible reasons are decreased symbiotic or asymbiotic N fixation, differences in residue decay dynamics, and short-term priming of soil organic matter mineralization leading to smaller long-term pools of available C and N. Alternatively, past N fertilization could lead to increased N immobilization in microbial biomass, resulting in less net N mineralization, although gross N mineralization may have been unaffected.

Both symbiotic and asymbiotic N fixation decline with increasing soluble inorganic N (Wilson, 1940; Knowles and Denike, 1974; Streeter, 1988; Stacey et al., 1992). Although preplant soil inorganic N in spring 1998 was unaffected by fertilization (Fig. 4), more inorganic N would have been available in soils after fertilizer application in LN and HN than in 0N. Thereby, LN or HN treatments could have reduced symbiotic and/or asym-

Table 4. Average annual stover and/or hay yield, C and N content of all non-grain biomass of all crops in continuous corn (CC), corn–soybean (CS), corn–soybean–wheat/alfalfa–alfalfa (CSWA) for one full rotation.†

Rotation	1997 Crop	Fertilizer N level‡	Stover or hay mass§	Stover or hay C	Stover or hay N	Weighted stover or hay C/N¶
			kg ha^{-1}			
CC	Corn	0N	4700 (520)	2040 (240)	18 (2.7)	111 (3.7)
		LN	6430 (520)	2820 (260)	34 (6.6)	82 (9.1)
		HN	7630 (460)	3380 (220)	51 (11)	67 (12)
CS	Soybean	0N	4200 (140)	1800 (80)	25 (7.6)	87 (6.4)
		LN	4950 (170)	2130 (58)	30 (0.9)	78 (6.2)
		HN	4820 (190)	2070 (78)	32 (5.2)	70 (6.6)
CSWA	Alfalfa	0N	5500 (240)	2400 (108)	93 (4.4)	53 (2.4)
		LN	5770 (160)	2530 (76)	102 (7.7)	44 (2.4)
		HN	5860 (190)	2570 (80)	100 (9.6)	43 (3.7)

† Values are means ($n = 3$) with standard deviation in parentheses, based on 1997 data for all plots.
‡ 0N, zero N fertilizer; LN, low N; HN, high N. Treatments 0N, LN, and HN fertilized for corn yield goal of 0, 5.3, and 8.5 Mg ha^{-1}, respectively.
§ Stover mass of corn and soybean is total aboveground biomass production excluding grain. Hay mass of alfalfa is sum of three cuttings during growing season.
¶ Weighted by proportion of total stover or hay production by each crop in rotation.

biotic N fixation and the supply of mineralizable N. However, in this study alfalfa received no fertilizer N in the fourth year of CSWA, although a small amount of N was applied at the time of wheat and alfalfa interseeding. Increased symbiotic N fixation in legumes in 0N plots could supply more mineralizable N, but only in legume rotations. Since the phenomenon appeared in CC, symbiotic N fixation is probably not the source of greater mineralizable N in all cases. In addition, total biomass, biomass N, and C/N ratio of alfalfa and soybean did not differ appreciably at different N fertilization levels (Table 3). Nonsymbiotic N-fixation is likely to be greater in low N treatments, but is generally considered a minor contributor to labile N pools in temperate agricultural soils (Lamb et al., 1987), too minor to explain the differences observed in this study. However, measurement of nonsymbiotic N fixation is rare in arable soils not fertilized with N.

Fertilization may have decreased the long-term supply of labile N through the priming effect. While the priming effect is still a subject of debate, recent work has documented changes in microbial activity after N additions. Application of N fertilizer (NH_4NO_3) can cause a temporary increase in microbial ammonification activity and specific respiration (Lovell and Hatch, 1998). Azam et al. (1995) induced increased mineralization of vetch (*Vicia* spp.) residues with addition of either NH_4 or NO_3. Woods et al. (1987) observed enhanced mineralization of soil N after addition of NH_4SO_4. An annual temporary rise in mineralization due to fertilization could increase the immediate supply of inorganic N but reduce the remaining supply of mineralizable materials in LN and HN plots.

Differences in residue decay dynamics could also produce increased mineralizable organic N in 0N treatments compared with LN or HN. On average, residues in 0N plots have a greater C/N ratio than residues in LN or HN plots (Table 4). This is primarily due to differences in corn residue C/N in CS and wheat residue C/N in CSWA (data not shown). Materials with C/N ratio larger than 40:1 decay slowly, and the decay rate is inversely related to C/N ratio (Vigil and Kissel, 1991). Slower residue decay in unfertilized plots could increase C and N pools with time. Some of the additional C and N would be mineralizable in the long term.

Mineralized N that is subsequently immobilized and incorporated in microbial biomass at the time of leaching will not be extracted or measured by periodic leaching methods. Although N immobilization was not measured during this incubation, the soil samples used in this incubation were found to have a negative relationship between past N fertilization and 10-d mineralizable C, dehydrogenase enzyme activity, and microbial biomass (by substrate-induced respiration) (data not presented). These findings suggest that N immobilization and gross N mineralization may have been even greater in unfertilized soils. However, avoidance of residues at sampling and the removal of high C/N residues from soil samples could have skewed the observed mineralization. If field residues, which have greatest C/N in 0N treatments, had been included in the soil samples more immobilization might have occurred, especially in 0N soils.

CONCLUSIONS

Variable-temperature incubation can be used to mimic soil temperature dynamics under a growing crop. When used with composite but minimally disturbed soil samples, VTI can provide both low sample variance and a small initial flush of mineralization while mimicking field soil temperature and its effects on mineralization.

After 8 yr, N mineralization was greater in CSWA than CS and CC. Rotation with legumes increased the N mineralizable from soil to support crop production. Alfalfa plow-down after 1.5 yr growth supplied 189 kg N ha^{-1}, 47 and 56 kg N ha^{-1} more N than previous crops of soybean or corn, respectively. Across rotations, net N mineralization was negatively affected by N fertilization. The average difference in net N mineralization in 0N plots compared with LN and HN plots was 16 kg N ha^{-1}. This suggests a small negative impact of N fertilization on labile N pools in the soil studied. While inorganic N is known to decrease both symbiotic and asymbiotic N fixation, crop data do not suggest greater symbiotic N fixation in 0N treatment. Yield and biomass N of crops were generally less in 0N treatments. Asymbiotic N fixation is generally considered negligible. Fertilizer N may alter the soil supply of labile N by reducing crop residue residence time in soil. Fertilizer addition may increase microbial activity temporarily, either directly after fertilization causing a priming of soil organic matter mineralization, or indirectly when large quantities of lower C/N ratio residues are incorporated into well-fertilized soils, leaving less mineralizable material for future use.

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