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Effects of management practices on annual net N-mineralization in a restored prairie and maize agroecosystems

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Abstract. Nitrogen (N) mineralization is a spatially variable and difficult component of the N cycle to quantify accurately under field conditions. Net N-mineralization was compared by direct measurement, indirect estimate, and laboratory incubation for a restored tallgrass prairie and for deficiently and optimally N-fertilized, no-tillage (NT) and chisel-plowed (CP) maize (*Zea mays* L.) agroecosystems on Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudoll) in Wisconsin, USA. Four years of *in-situ* field measurements using an incubated-soil-core/ion-exchange-resin-bag technique showed that land use significantly affected net N-mineralization. Net N-mineralization was consistently smaller in the restored prairie than in the maize agroecosystems and typically larger in the CP than in the NT maize agroecosystems. Three independent methods for indirectly estimating annual net N-mineralization (i.e., N budget residual, deficiently N-fertilized plant N uptake, and profile-scaled *in-situ* field measurements) were relatively consistent at capturing land-use and tillage effects on net N-mineralization. Laboratory incubation and periodic leaching of Fall-sampled soils demonstrated that both mineralized N and labile C were co-limiting factors influencing N-mineralization in agricultural soils and generally supported field measurements by showing a significant difference in net N-mineralization with and without added fertilizer-N.

Introduction

Nitrogen (N) is the nutrient most often limiting maize (*Zea mays* L.) production in the Midwestern United States. Mineralization is an important source of N for crops and this source of N should be considered by farmers when determining crop nutrient requirements. Mineralized-N in unfertilized maize contributes roughly 0.9 kg N for every 900 kg of soil organic matter to maize production in humid to subhumid, silt loam soils, which is equivalent to ~40 to 50% of the mineral-N uptake in a N-fertilized or N-unfertilized maize crop (Schulte and Walsh 1998). Adequately quantifying the supply of mineralized-N to a crop throughout the growing season improves the likelihood that commercial N fertilizer is applied at rates sufficient to meet plant needs without excess that may cause surface or groundwater contamination (Mahmoudjafari et al. 1997; Wienhold and Halvorson 1999).

Nitrogen mineralization is most often referred to as the net sum of two simultaneously occurring, microorganism-mediated processes: 1) the release of N by ammonification and 2) the uptake through N-immobilization. Ammonification releases mineral ammonium-N (NH_4^+-N) from soil organic matter, while nitrification converts NH_4^+-N to mineral nitrate-N (NO_3^--N). Immobilization is the processes by which microorganisms take up mineral N from the soil while consuming carbon (C)-rich organic matter substrate. Microorganisms both consume mineral-N and release it to the soil (i.e., gross immobilization and mineralization, respectively), with mineralized-N either re-used by other microorganisms (i.e., net immobilization) or residing in the soil (i.e., net mineralization) if not leached out. Net mineralized-N supplies most N to vegetation, except for N-fixing legumes and agricultural crops provided more N for optimal yields. Organic material that is returned to the soil surface as residues becomes the substrate for microbial activity, which controls both C and N cycling in natural and managed ecosystems. Consequently, N-mineralization is influenced by past land use (Thorne and Hamburg 1985), residue management practices, and environmental factors that alter microbial activity and N use (Linn and Doran 1984; Jarvis et al. 1996).

Vegetation and land use, such as forest, prairie, pasture, or cultivated agriculture, affect soil physical, chemical, and biological properties and biogeochemical processes. Residue or leaf litter that is returned to the soil throughout the growing season or following harvest of a crop is an important source of C for microbes. Mixing the soil through mechanical tillage stimulates microbial activity in the top 5 to 20 cm by incorporating organic matter into the soil, improving soil aeration, disrupting aggregates, and increasing surface area for decomposition, all of which in turn accelerate oxidation of organic material and increase soil carbon dioxide (CO_2) flux. Increased microbial C cycling increases N cycling because of low C:N ratios in microbes.

Soil C and N dynamics, especially N-mineralization rates, are spatially and temporally variable in soils making them difficult to measure or model (Jarvis et al. 1996). Net N-mineralization estimates for natural and managed ecosystems range from < 1 kg N ha^{-1} mo^{-1} to 129 kg N ha^{-1} yr^{-1} using an incubated-soil-core/ion-exchange-resin-bag approach, from 62 kg N ha^{-1} growing $season^{-1}$ to 1140 kg N ha^{-1} yr^{-1} using the buried bag approach (Eno 1960), and from 13 kg N ha^{-1} $(40\ wk)^{-1}$ to 394 kg N ha^{-1} yr^{-1} using an incubation-with-periodic-calcium-chloride($CaCl_2$)-leaching technique (Table 1).

In a review of our understanding of N-mineralization in temperate soils, Jarvis et al. (1996) recognized that better quantification of the effects of land use, management practices, and controlling factors on N-mineralization, through technique improvements and long-term N-budget studies, would allow for improved management. Therefore, the specific objectives of this study were twofold: i) compare direct and indirect annual net N-mineralization estimations in natural and managed ecosystems of south-central Wisconsin, USA, and ii) determine the precision of

Table 1. Summary of nitrogen mineralization rates on an annual basis and for other time periods using various measurements techniques for selected agricultural, prairie, and forested ecosystems.

Ecosystem	Soil Type	Time Period	N-min Estimate kg N ha^{-1} period^{-1}	Measurement Technique	Reference
Dryland fallow	loam	April 30-July 22	33.7	*In situ* soil core/ion exchange resin	Kolberg et al. (1997)
	clay loam	April 30-July 15	26.5		
Fertilized corn agroecosystem	sandy silt	Aug. 10-Sep. 26	0.82	*In situ* soil core/anion exchange resin	Hübner et al. (1991)
Sedge birch-pine fen	peat	May-September	63–94	*In situ* soil core/ion exchange resin	Regina et al. (1998)
		Annual	87–129		
Fir stand		Annual	31–112	*In situ* soil core/ion exchange resin	Sasser and Binkley (1989)
Alder/Douglas fir stand		Annual	0.5–126	*In situ* soil core/ion exchange resin	Binkley et al. (1992)
Conifer stand	Alfisol	Annual	60	Buried bag and *in situ* soil core incubation	Reich et al. (1997)
Mesic hardwoods stand			60–110		
Oak stand			55–105		
Beech stand		Annual	24–238	Buried bag incubation	Ellenberg (1977)
Maple/Ash stand			100–173		
Ash/Maple stand			147–377		
Tropical hardwoods stand		Annual	128–634	Buried bag incubation	Pfadenhauer (1979)
Tropical hardwoods stand		Annual	588–1140	Buried bag incubation	Matson et al. (1987)

Table 1. Continued.

Ecosystem	Soil Type	Time Period	N-min Estimate kg N ha^{-1} $period^{-1}$	Measurement Technique	Reference
Prairie marsh	loam	Growing Season	124	Buried bag incubation	Neill (1995)
	sandy loam		62		
Wheat-fallow rotation		2 weeks	13–19	Aerobic incubation/ anion exchange membranes	Qian and Schoenau (1995)
Minimum tillage agroecosystem			17–20		
Native shortgrass prairie			19–20		
Uncultivated sugarcane	muck	Annual	149–348	Incubation in column lysimeters with periodic $CaCl_2$ leaching	Hanlon et al. (1997)
Cultivated sugarcane			13–221		
Sod			63–234		
Cultivated sweet corn			18–123		
Konza tallgrass prairie	silt loam	40 weeks	394	Incubation with periodic $CaCl_2$ leaching	Ajwa et al. (1998)
Cultivated winter wheat			153		
Wheat-corn-millet-fallow rotation	loam	30 days	32–52	Incubation	Wood et al. (1990)
	loam-clay loam		39–73		
	sandy loam-sandy clay loam		13–47		

three indirect methods for quantifying annual net N-mineralization using measurements from five land-use treatments over four years.

South-central Wisconsin is located near the northern boundary of the maize belt in the Midwestern United States. Mold-board or chisel-plow cultivation have been the traditional agricultural tillage practices used in this region, but no-tillage agriculture has gained wide acceptance. Similarly, high crop yields are dependent on N-fertilization practices, which can vary from low N, to optimal, to excessive N inputs. South-central Wisconsin is also located in an ecotone transition zone between grassland-dominated vegetation to the south and deciduous forest-dominated vegetation to the north. Consequently, remnant and restored prairies exist sporadically across the landscape, but offer a unique opportunity to use as a baseline for evaluating the effects of mechanized agriculture on biogeochemical processes such as net N-mineralization. This study aims to address a number of current research needs towards improving our understanding of N-mineralization in natural (i.e., restored prairie) and managed (i.e., optimally and deficiently N-fertilized no-tillage and chisel-plowed maize) ecosystems. The restored prairie and optimally and deficiently N-fertilized no-tillage and chisel-plowed maize agroecosystems were chosen for this comparative study because they are representative land uses for a large area in the Midwestern United States. Thus, the hypotheses for this study include that net N-mineralization is i) higher in cultivated agriculture than in restored prairie, ii) higher in chisel-plowed than in no-tillage agriculture, iii) higher in residue-amended than in residue-unamended soil, and iv) higher in optimally N-fertilized than in deficiently N-fertilized agricultural soil.

Materials and methods

Experimental site and design

Two study sites, a maize agroecosystem and a restored tallgrass prairie, were selected in May 1995 (Brye 1997). The agricultural site is located at the University of Wisconsin-Madison's Arlington Agricultural Research Station, Arlington, WI (43° 17′ N, 89° 22′ W). The prairie is located at the Audubon Society's Goose Pond Sanctuary north of the research station at Arlington, WI. Both sites reside on Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) and are geographically separated by < 2.5 km. Mean annual precipitation for the Arlington region is 790 mm, of which ~30% of annual precipitation occurs as snow, while the mean annual temperature for the region is 7.6 ºC with a January minimum of −13.8 °C and a July maximum of 28.3 °C (Owenby and Ezell 1992).

A randomized complete-block design replicated four times was established for conventional chisel-plowed (CP) and no-tillage (NT) maize treatments, with tillage-plot dimensions of 9.1 by 12.2 m (Brye et al. 2000). A 105-day relative maturity hybrid maize variety was planted for each tillage treatment at two fertilizer rates to represent optimal and deficient N levels for maize (Kelling et al. 1991). The fer-

Table 2. Initial site characteristics for the restored prairie and maize agroecosystems adapted from Table 2 of Wagai et al. (1998). Standard errors are reported in parentheses for replicate measurements.

Site Characteristics	Prairie	Maize Agroecosystems
Physiography		
Elevation (m)	305	305
Slope (%)	3	2
Slope position	back slope	back slope
Aspect	NW	NNE
Soil Properties		
Taxonomy	Typic Argiudoll	Typic Argiudoll
Parent material	loess over till	loess over till
Sand fraction	0.14	0.16
Silt fraction	0.60	0.59
Clay fraction	0.26	0.25
Bulk density$_{30\ \mathrm{cm}}$ (g cm^{-3})	1.33	1.34
Organic matter$_{0\text{–}30\ \mathrm{cm}}$ (%)	3.1 (0.1)	3.3 (< 0.1)

tilizer-rate treatments were superimposed as a split-plot design on the randomized complete-block design for the tillage treatments. Optimally N-fertilized tillage treatment combinations, 6.1 × 12.2 m, received 189 kg N ha^{-1} yr^{-1}, while deficiently N-fertilized tillage treatment combinations, 3.0 × 12.2 m, received 10 kg N ha^{-1} yr^{-1} as a starter fertilizer. Nitrogen fertilizer was delivered as uniformly as possible by hand in a broadcast application of pelletized ammonium nitrate (NH_4NO_3) immediately following planting.

Four 7- × 7 -m plots were established at the natural prairie in spring 1995 (Brye et al. 2000). Historically, the prairie was subject to tillage and fertilization practices similar to those used on the Arlington Agricultural Research Station. The prairie was restored from agricultural use in June 1976 by re-seeding to native tallgrasses [i.e., big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* (L.) Nash), and switchgrass (*Panicum virgatum* L.)] and forbs [i.e., purple coneflower (*Echinacea purpurea*), goldenrod (*Solidago spp.*), and black-eyed susan (*Rudbeckia hirta*)]. The current vegetation is mesic tallgrass prairie. The prairie is typically burned on a three-year cycle and was last burned on 18 April 1998. Table 2 lists physiographic and soil similarities between the prairie and agricultural site. A more detailed description of the sites can be found in Brye (1997) and Wagai et al. (1998).

Environmental conditions

Chromel-constantan insulated thermocouples (Omega Engineering, Inc.; Stamford, CT) embedded in multi-purpose RTV sealant (Dow Corning; Midland, MI) and protected by 5.1 cm of 6.3 -mm diameter copper tubing were fabricated for soil temperature measurements at 10-, 30-, 70-, and 120 -cm at each study site. A micrometeorological weather station exists on the Arlington Research Station ~275 m from the agricultural site. Data recorded at this station were used for the agricultural site, while precipitation, air and soil temperatures, and relative humidity were also measured at the prairie site.

Soil inorganic N

Soil samples 2 -cm in diameter were collected from the top 30 cm at 10-day (d) intervals throughout the growing season, typically between April and October, in the prairie and deficiently N-fertilized agroecosystems from 1995 through 1998 to assess relative differences in soil mineral-N content over time. Two soil cores were collected per plot, thoroughly mixed, composited, and dried immediately. Dry-soil samples were ground with gentle compression and sieved through a 2 -mm mesh screen. Dried soil, 2.5 g, was extracted with 25 mL of 2 M potassium chloride (KCl) and shaken for 1 hr (Bundy and Meisinger 1994). Additionally, a sub-set (n = 16) of fresh soil samples (~15 g) was collected on one of the 10 -d sampling intervals in 1996 to assess the impact of drying on the mineral-N content since the effects of drying soil are known to be large. Fresh soil was immediately extracted in the field in 100 mL of 2 M KCl, shaken for 1 hr, and allowed to settle over night to compare extractable inorganic N in fresh- versus dried-soil samples. The KCl-soil solutions were filtered through Whatman G6 glass fiber filter paper. A subsample of the filtrate was collected and refrigerated for generally < 7 d, during which time the samples were assumed stable prior to and for chemical analysis. Colorimetric analysis for NO_3^-- and NH_4^+-N was performed for all KCl-soil extracts using a Lachat continuous-flow ion analyzer (Lachat Instruments (1986, 1987)). Inorganic soil N content was calculated from inorganic soil N concentrations and bulk density measurements of 1.33 [standard error (SE) ± < 0.1], 1.34 (SE ± < 0.1), and 1.31 (SE ± < 0.1) g cm^{-3} for the prairie, NT, and CP ecosystems, respectively (Brye 1999; Kucharik et al. 2001).

In-situ *net N-mineralization measurements*

In-situ net N-mineralization was measured in the top 20 cm, roughly corresponding to the plow layer, monthly throughout the growing seasons of 1995 through 1998 using an in-situ-soil-core/ion-exchange-resin-bag (ISC/IERB) technique similar to the method of DiStefano and Gholz (1986). Two, 20 -cm long soil cores were collected in poly-vinyl chloride (PVC) tubes, 7.6 cm in diameter by 30 cm long, from the furrows without wheel tracks within each optimally and deficiently N-fertilized, NT and CP plot (i.e., n = 8 cores per treatment) immediately after planting, which typically occurred between 24 April and 7 May. The PVC tubes were sharpened with a bevel to the outside at the base to minimize soil compaction in the core during installation. Small bags, consisting of an even mixture of anion and cation exchange resin beads (J.T. Baker Inc., Phillsburg, NJ., 08865; Anion Exchange Resin #4601–01; Cation Exchange Resin #1927–01) were placed at the base of the soil core as it was placed back into its original position and left to incubate *in-situ*.

Soil samples were collected from the 0 to 20 cm depth outside the PVC tube and a 15 -g subsample of fresh soil was immediately extracted with 100 mL of 2 M KCl for initial NO_3^--N and NH_4^+-N determinations. After incubating for approximately 1 mo, the PVC-cores were collected from the field plots. The ion exchange resin bags were immediately extracted in 100 mL of 2 M KCl. The soil in the PVC tube was removed, homogenized by hand, and approximately 15 g of fresh soil was extracted in 100 mL of 2 M KCl for final NO_3^--N and NH_4^+-N determinations. Details of the ISC/IERB method are described in Brye et al. (2002b). Net N-mineralization (N_{MIN}) was calculated using the following equation,

$$[(NO_3^- - N + NH_4^+ - N)_{FINAL} - (NO_3^- - \mathrm{N} + NH_4^+ - N)_{INITIAL}] + (NO_3^- - N + NH_4^+ - N)_{RESIN} = N_{MIN}.$$

Gravimetric soil moisture content was also determined on pre- and post-incubation soil samples.

Monthly net N-mineralization rates measured in the top 20 cm were also scaled to the soil profile by assuming that net N-mineralization rates were proportional to soil profile C for the four agroecosystems (Brye 1999). A C source must exist for microorganisms to perform functions such as mineralization. Since soil C typically decreases exponentially with depth, a reasonable assumption would be that microbial activity decreases concomitantly with soil C. Profile-scaling factors were calculated based on the mean fraction of soil C that existed in the top 20 cm relative to the soil C in the rest of the profile (i.e., 120 cm). Therefore, net N-mineralization rates were assumed to have the same profile-scaling factor as for soil C. The profile-scaling factors used to scale net N-mineralization estimates from the top 20 cm to the top 120 cm were 2.2 for the prairie, 2.7 for the deficiently N-fertilized no-tillage, and 2.8 for the deficiently N-fertilized chisel-plowed and both fertilized tillage treatments.

Indirect estimates of net N-mineralization

Net N-mineralization was also indirectly estimated by two additional methods: 1) as the residual of an ecosystem N budget, and 2) as being equivalent to deficiently N-fertilized plant N uptake. In a related study, the N budget was constructed for the prairie and maize agroecosystems from independent field measurements of the major components of the N budget (Brye 1999; Brye et al. 2002c (in press)). The residual term of the N budget was assumed to equal the net effect of N-mineralization and denitrification. However, deep soil denitrification (i.e., at 1.4 m) was negligible for the prairie and agroecosystems (Brye et al. 2001); therefore, the residual term from the N budget was assumed here to be an indirect estimate of net N-mineralization. The N-budget approach has been used before to determine N-mineralization in field studies (Powlson et al. 1986; Vinten et al. 1992).

Plant N uptake from the prairie and the deficiently N-fertilized maize tillage treatments was assumed equal to mineralized-N extracted from the soil by the plant during the growing season. Whole plant samples were collected from the prairie at maximum green leaf area index (i.e., approximately late August) and from the agroecosystems at physiological maturity (i.e., between mid-September and mid-October). Plant samples were dried for 48 hrs at 63 °C and ground to pass a #60-mesh screen. Total plant N was determined on ground tissue by a micro-Kjeldahl digestion procedure (Nelson and Sommers 1973; Lachat Instruments 1993). The plant-N-uptake-estimate method assumes that the vegetation extracts all of the N that becomes available by net N-mineralization and that N-mineralization is unaffected by potential fertilizer-N rate differences. The plant-N-uptake method has also been widely used to estimate net N-mineralization in greenhouse and field experiments (Jarvis et al. 1996).

N and C mineralization by laboratory incubation

Net N-mineralization was measured in the laboratory by the method of Bundy and Meisinger (1994) for soil samples collected from the field plots in 1998. Soil samples were collected in the Fall, following the growing season, while inorganic N and labile C were assumed depleted relative to their status at the beginning of the growing season, to maximize potential treatment effects. Treatments were chosen to evaluate how N-mineralization is affected by i) three Upper-Midwestern USA land uses (i.e., prairie and fertilized and deficiently N-fertilized CP maize soil) with residue, but without fertilizer, and ii) residue and N additions to incubated soil within one wide-spread cropland management treatment (i.e., optimally N-fertilized, CP maize soil).

Approximately 70 g fresh soil was incubated in small incubation cups and periodically leached under vacuum with 100 mLs of 0.01 M $CaCl_2$. Five replicate samples of six soil treatments with various fertilizer and residue combinations were incubated: i) prairie soil with residue and without fertilizer (Trt 1), ii) deficiently N-fertilized CP soil with residue and without fertilizer (Trt 2), iii) optimally N-fertilized CP soil with residue and fertilizer (Trt 3), iv) optimally N-fertilized CP soil with residue and without fertilizer (Trt 4), v) optimally N-fertilized CP soil with fertilizer and without residue (Trt 5), and vi) optimally N-fertilized CP soil without residue or fertilizer (Trt 6). Thus the treatments included CP soil with a history of optimal N-fertilization as well as soil with a history of deficient N-fertilization; in addition, CP soils were fertilized and not fertilized during the laboratory incubation.

Each soil treatment received its own ground residue (i.e., $\leqslant$ #60-mesh size) equivalent to the average amount of residue on the soil surface of each of the three soil treatments from the previous four years (i.e., 0.34, 0.77, and 1.39 g residue were added to the prairie, deficiently, and optimally N-fertilized CP treatments, respectively). Residue was left on the surface of the prairie soil treatment and incorporated by mixing into the agricultural soil treatments. Incubation cups were prepared by packing fresh soil to a bulk density of 1.0 g cm^{-3}. (A bulk density greater than this would have not allowed leachate solution to flow at a reasonable rate through the incubation cups.) The two fertilized treatments (i.e., Trt 3 and 5) received 0.191 g N (incubation cup)$^{-1}$ as NH_4NO_3 in ~5 mL of solution, which was equivalent, on an areal basis, to the rate of N applied to the field plots (i.e., 189 kg N ha^{-1}). Initial gravimetric soil moisture was near field capacity, between 15 and 18%, for the soil treatments. Soil samples were leached with $CaCl_2$ after 7, 15, 30, 60, 90, and 120 d.

After leaching, soil samples received 25 mLs of a < 0.005 M N-free nutrient solution containing calcium, sulfur, magnesium, phosphorus, and potassium (Bundy and Meisinger 1994). Incubating soil cups were then drawn under vacuum to a uniform moisture potential (~60 kPa) and stored in the dark at 25 °C between leachings. Gravimetric soil moisture content in the incubation cups was re-adjusted weekly to near field capacity by adding water until a target mass for each incubation cup was reached. Inorganic N status was determined for pre- and post-incubation soil by KCl extraction. We assumed that a significant portion of the added fertilizer N would be immobilized by microorganisms within the first 5 to 7 d and that inorganic N captured in the leachate solutions represented either re-mobilized fertilizer N or mineralized-N from native organic matter, in which the sum of both was identified as net N-mineralization. Therefore, net N-mineralization was calculated as the difference between final and initial soil inorganic N contents (i.e., prior to N addition in the fertilized treatments) plus leached inorganic N for the duration of the 120 -d incubation period.

Soil CO_2 flux was also measured on all incubating soil cups twice per week throughout the 120 -d incubation. Soil cups were removed from the temperature-controlled incubation chamber and placed into 1.7 L plastic bottles and sealed. An initial ambient CO_2 concentration measurement was taken from each sealed bottle using a Li-Cor LI-6262 CO_2 analyzer (Li-Cor, Lincoln, NE). A pump circulated the atmosphere from within the bottles through the CO_2 analyzer for 10 s and the CO_2 concentration was recorded 15 s after the pump was shut off. After the initial ambient CO_2 reading, the soil cups were sealed and incubated for ~1 hr (± 5 minutes) while a small 12 V DC-powered mini-fan (model 2510-12M; Pacific Electronics, Crystal Lake, IL), mounted to hang from the under-side of each bottle's plastic cap, gently mixed the air inside the bottle containing the incubating soil cup. The final CO_2 concentration reading was then measured. Soil CO_2 flux was calculated as the change in CO_2 concentration over the total incubation time.

Two N-mineralization indices were calculated from the laboratory incubation data. The first was calculated as the ratio of cumulative leachate N and cumulative C evolved between leachings, while the second was calculated as the ratio of net N-mineralization and total C evolved during the entire incubation period. These indices are used to demonstrate the coupling of C dynamics with N dynamics and how land use and management practices affect this coupling.

Statistical analyses

Comparative studies, such as this study, cannot be used to illustrate cause and effect, but do provide the opportunity to investigate the effect of land use on ecosystem structure and function (Wagai et al. 1998). To infer a land use effect on soil and vegetation properties using a comparative study, the initial site and soil conditions must be as uniform as possible. Numerous lines of evidence, such as similar physiography, climate, soil series, and selected soil physical and chemical characteristics (Table 2), suggest that our underlying assumption (i.e., that the prairie and agroecosystems are uniform and the comparison is valid) is reasonable. This analysis assumes the variability among the four replicate plots of each treatment is not atypical of the variability that would be expected among plots in general that share similar soil, ecological, and climate conditions (Wagai et al. 1998). Therefore, a one-way analysis of variance (ANOVA) was performed, assuming a randomized experimental design (i.e., without accounting for blocking of the maize tillage treatments), to determine ecosystem treatment differences for net N-mineralization estimates for individual sampling dates (SAS Institute Inc 1990). Coefficients of variation were also calculated for comparing net N-mineralization estimates across methods.

Results

Inorganic soil N and net N-mineralization

Mean soil NO_3^--N in the top 30 cm ranged from 9.5 to 17.4 kg ha^{-1} for the deficiently N-fertilized NT maize agroecosystem and from 11.0 to 19.3 kg ha^{-1} for the deficiently N-fertilized CP maize agroecosystem (Figure 1). Mean soil NO_3^--N in the top 30 cm for the entire growing season was significantly greater ($p < 0.001$) for the deficiently N-fertilized maize agroecosystems than for the prairie, which ranged from 1.6 to 3.3 kg NO_3^--N ha^{-1} between 1995 and 1998 (Figure 1). Soil NH_4^+-N in the top 30 cm was similar from date to date among the prairie and deficiently N-fertilized maize agroecosystems within a given year, but steadily decreased between 1995 and 1998 for all three ecosystems (Figure 2). Between 1995 and 1998, mean extractable NH_4^+-N was 9.1–35.9, 7.4–31.5, and 7.9–35.4 kg ha^{-1} for the prairie and deficiently N-fertilized NT and CP maize agroecosystems, respectively (Figure 2). Generally, the available soil-N pool (i.e., NO_3^--N + NH_4^+-N) in the restored prairie was less than in the agroecosystems, though statistical significance was not evaluated. Extractable inorganic N was also significantly greater ($p < 0.001$) for dried (341 kg N ha^{-1}) than fresh soil samples (183 kg N ha^{-1}), where the mean difference was 158 kg N ha^{-1}.

Monthly *in-situ* net N-mineralization estimates using the ISC/IERB method were significantly different ($p < 0.05$) among the five ecosystem treatments for 3 of 3 dates in 1995, 5 of 6 dates in 1996, 3 of 6 dates in 1997, and 2 of 6 dates in 1998

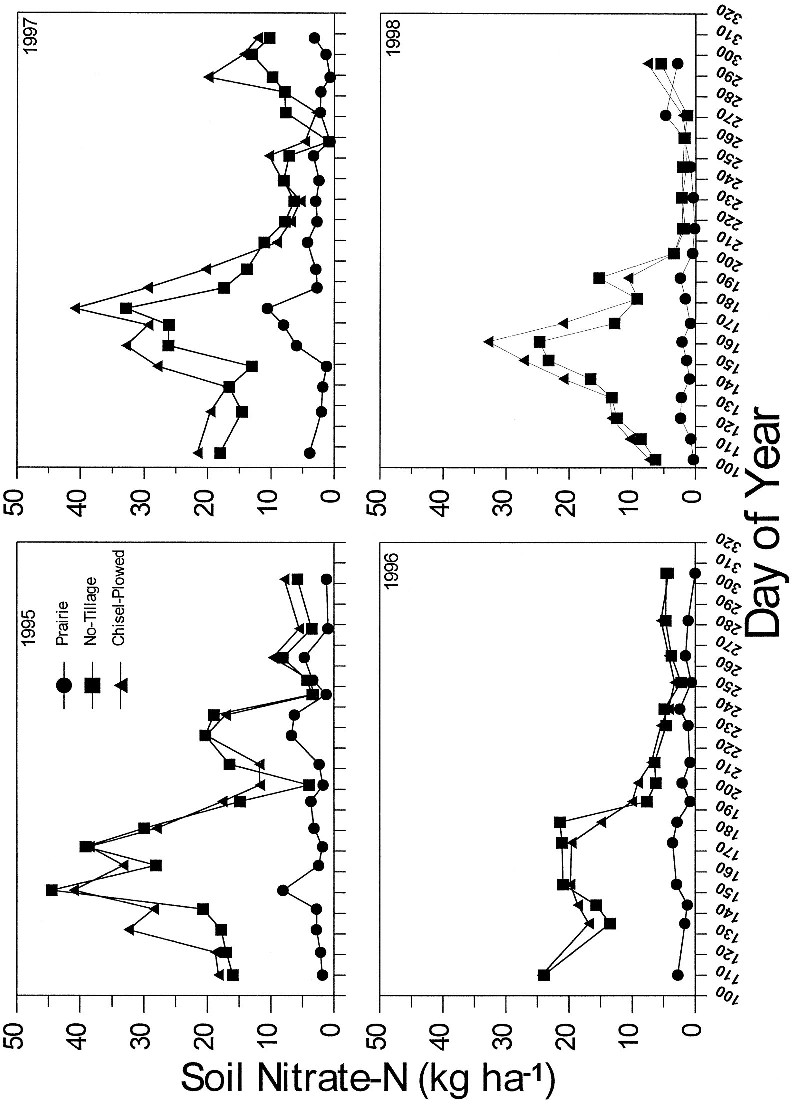

Figure 1. Soil nitrate-N in the top 30 cm for the prairie and deficiently N-fertilized no-tillage and chisel-plowed maize agroecosystems between April and October for 1995 through 1998.

(Figure 3). Mean net N-mineralization was generally smaller and less variable in the restored tallgrass prairie than in the maize agroecosystems, but the effects of tillage and fertilization were generally inconsistent over the growing season within a given year. No one maize agroecosystem consistently maintained the highest net N-mineralization for each sampling within a growing season or between years. Over the course of four growing seasons, net N-mineralization ranged from −11 to 7, −5 to 58, and −13 to 80 kg N ha^{-1} yr^{-1} in the prairie and deficiently and optimally

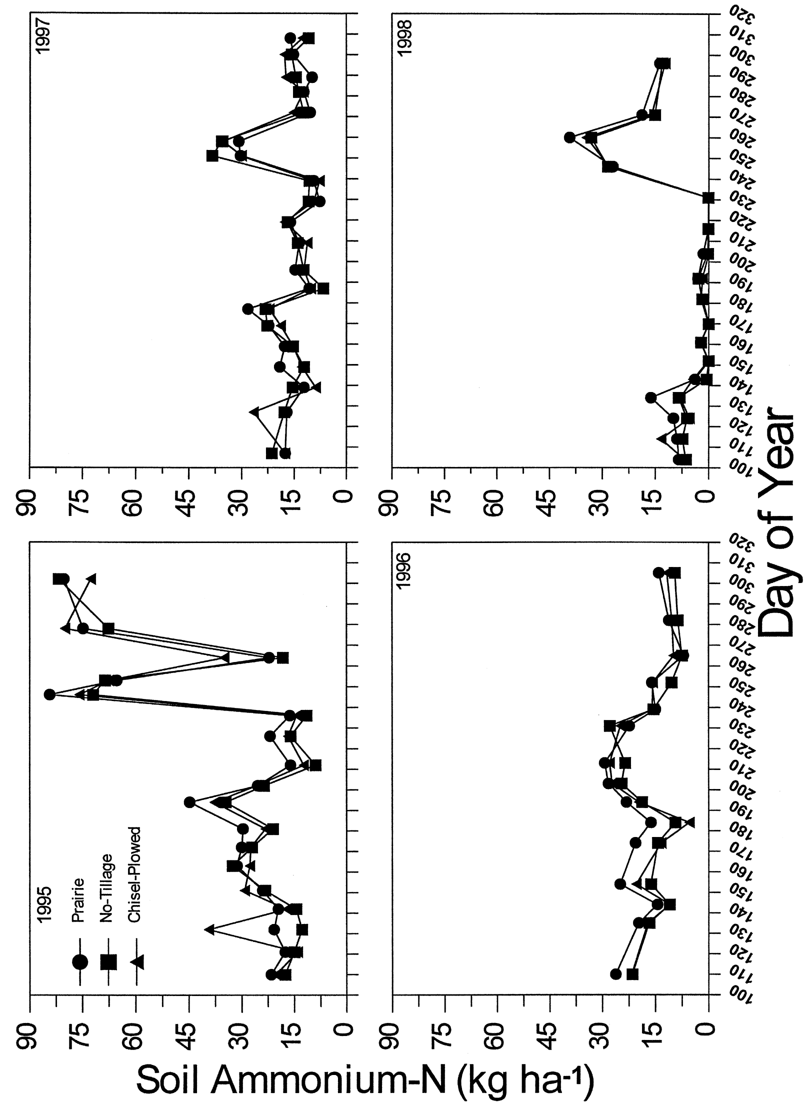

Figure 2. Soil ammonium-N in the top 30 cm for the prairie and deficiently N-fertilized no-tillage and chisel-plowed maize agroecosystems between April and October 1995 through 1998.

N-fertilized maize agroecosystems, respectively (Table 3). Similarly, growing-season net N-mineralization ranged from −13 to 55 kg N ha^{-1} yr^{-1} for the NT maize and from −5 to 80 kg N ha^{-1} yr^{-1} for the CP maize agroecosystems. Mean growing season net N-mineralization was −3, 34, 34, 18, and 61 kg N ha^{-1} yr^{-1} for the prairie, deficiently N-fertilized NT and CP, and optimally N-fertilized NT and CP, respectively.

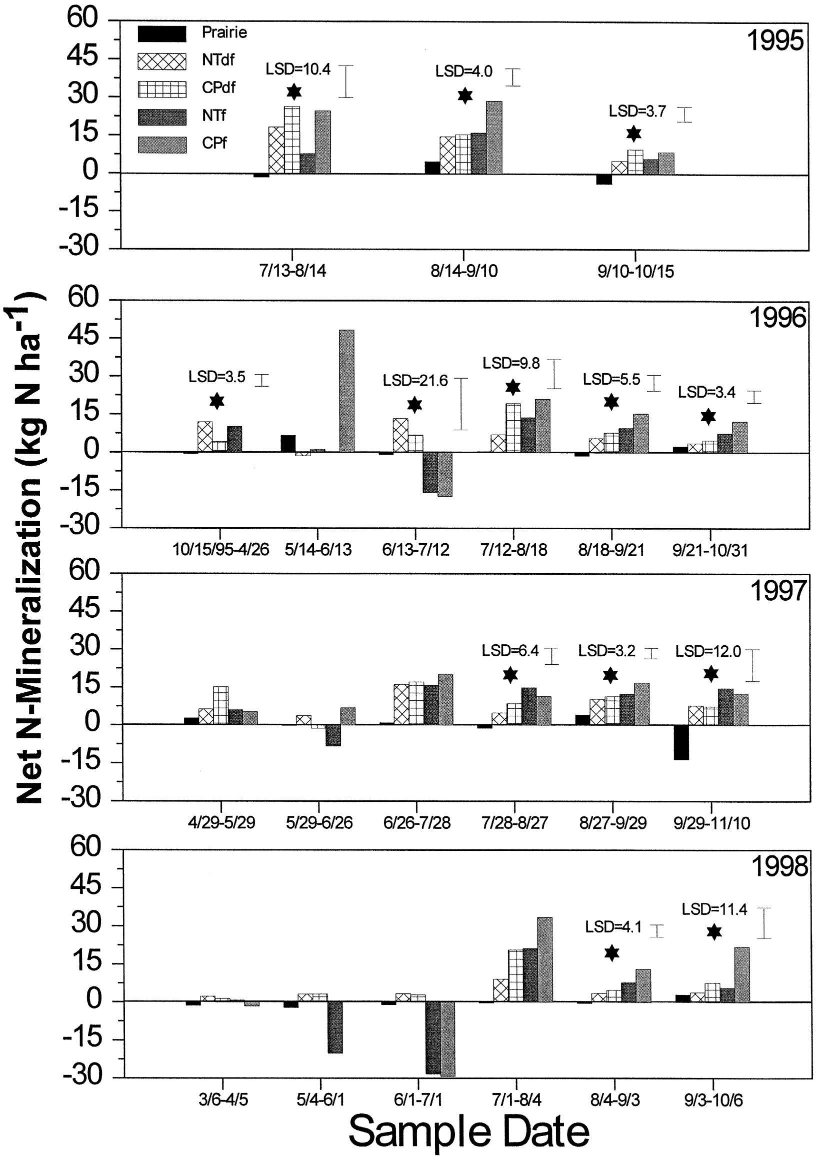

Figure 3. Net N-mineralization measured in the field with the ISC/IERB technique for the prairie, deficiently N-fertilized no-tillage (NTdf) and chisel-plowed (CPdf), and optimally N-fertilized no-tillage (NTf) and chisel-plowed (CPf) agroecosystems throughout the growing season from 1995 through 1998. Significant treatment differences ($p < 0.05$) within a incubation period are denoted by a star. Least significant differences (LSDs) are provided for significant treatment effects ($\alpha = 0.05$).

Indirect estimates of annual net N-mineralization varied by method (i.e., residual from the N budget, deficiently N-fertilized plant N uptake, and summation of direct *in-situ* field measurements scaled to the profile) and year (Table 3). The rank order

Table 3. Comparison among annual net nitrogen (N) mineralization estimates by four methods: 1) the residual from the N budget, 2) the equivalent to the deficiently N-fertilized plant N uptake, 3) *in-situ* field measurements, and 4) profile-scaled *in-situ* net N-mineralization estimates.

Year/Ecosystem	Annual Net N-mineralization Estimate Method				Mean N-Min[¶]
	N-Budget Residual	Deficiently N-fertilized Plant N Uptake	*In-situ* Field Measure-ments(0 to 0.2 m)	Profile-Scaled *In-situ* Field Measure-ments(0 to 1.2 m)	
	kg ha^{-1} yr^{-1}				
1995					
Prairie	33	18 (1) [†]	−11 (3.1) [‡]	−25	8.3
Deficiently N-fertilized NT	–	113 (6)	35 (7.1)	96	–
Deficiently N-fertilized CP	–	114 (11)	−5.1 (12)	−14	–
Optimally N-fertilized NT	–	–	14 (4.6)	38	–
Optimally N-fertilized CP	–	–	30 (11)	83	–
1996					
Prairie	0.2	7.5 (1.3)	7.4 (4.1)	17	8.1
Deficiently N-fertilized NT	–	88 (8.9)	28 (5.8)	77	–
Deficiently N-fertilized CP	–	85 (5.4)	40 (7.1)	113	–
Optimally N-fertilized NT	66	–	15 (17)	42	65
Optimally N-fertilized CP	111	–	80 (49)	224	140
1997					
Prairie	42	15 (3.1)	−7.1 (9.0)	−16	13
Deficiently N-fertilized NT	–	122 (9.0)	49 (7.1)	134	–
Deficiently N-fertilized CP	–	152 (7.3)	58 (11)	164	–
Optimally N-fertilized NT	224	–	55 (14)	153	166
Optimally N-fertilized CP	218	–	73 (32)	205	192
1998					
Prairie	77	17 (1.5)	−2.5 (3.5)	−5.6	30
Deficiently N-fertilized NT	–	78 (5.5)	25 (2.4)	69	–
Deficiently N-fertilized CP	–	95 (5.6)	41 (3.9)	115	–
Optimally N-fertilized NT	2.5	–	−13 (42)	−36	15
Optimally N-fertilized CP	25	–	–	–	60

[†] Standard errors are provided in parentheses for the deficiently N-fertilized plant N uptake data.

[‡] Probable errors, calculated from the square root of the sum of the squares of the standard error for each monthly measurements, are provided in parentheses for the *in-situ* field measurements from 0 to 0.2 m.

[¶] Mean N-mineralization (N-min) was calculated for only those treatments where estimates from the N-budget residual, plant N uptake, and profile-scaled methods were available. Plant N uptake values for the deficiently N-fertilized treatments were used for the optimally N-fertilized treatments.

of annual net N-mineralization estimates varied somewhat for different land uses within the same year and across years. The CV for the different methods ranged from 101 to 360% for the prairie, but net N-mineralization values were usually much smaller in magnitude for the prairie than net N-mineralization values for the agroecosystems. The CV ranged from 5 to 82% for the maize agroecosystems, except for the deficiently N-fertilized CP treatment in 1995 and the optimally N-fertilized NT treatment in 1998 when the profile-scaled net N-mineralization estimates were negative compared to all positive values from the other indirect estimates. With a few exceptions, patterns of variation in net N-mineralization estimates, averaged across methods, were roughly consistent in terms of both rank order and magnitude. Regardless of method, net N-mineralization in the restored prairie was generally smaller than in the maize agroecosystems, which was expected since natural ecosystems that are uncultivated and unfertilized are frequently N-limited. Similarly, net N-mineralization was generally greater in the CP than NT maize agroecosystems, but the effect of fertilizer-N rate was inconsistent.

N and C mineralization by laboratory incubation

Laboratory incubations generally supported field measurements with respect to the effect of land use on net N-mineralization, but also showed a significant difference in net N-mineralization with and without added fertilizer-N ($p < 0.001$) (Figure 4b). Mineralized-N leached from the soil was greater for the residue-amended fertilized (Trt 3) than for the residue-amended deficiently N-fertilized soil (Trt 4) (Figure 4a). Similarly, mineralized-N leached from the soil was greater at all leaching intervals for the residue-unamended fertilized soil (Trt 5) than for the residue-unamended deficiently N-fertilized soil (Trt 6).

A potentially labile C source (i.e., incorporated residue) was unavailable to microbes in the residue-unamended fertilized soil (Trt 5) compared to the residue-amended fertilized (Trt 3) soil. Inorganic N in the leachate of Trt 5 was probably largely due to fertilizer-N passing through the soil column early into the incubation period (i.e., within 0 to 7 d) presumably because immobilization of the fertilizer-N did not occur in the absence of a labile C source (Figure 4a). Conversely, the amount of inorganic N in the leachate of Trt 3 was less than half that of Trt 5 at the day-7 leaching, suggesting that fertilizer-N was immobilized in Trt 3 and re-released as mineralized-N by the day-15 leaching and subsequent intervals (Figure 4a).

Soil respiration peaked by 8 d into the incubation for the six soil treatments. The two treatments using fertilized CP soil with residue (i.e., Trt 3 and 4, with and without fertilizer, respectively) respired the most C, while the two treatments using fertilized CP soil without residue (i.e., Trt 5 and Trt 6, with and without fertilizer, respectively) respired the least amount of C during the 120 -d incubation period (Figure 4b). The unamended treatments using fertilized CP soil (Trt 6) also had the smallest net N-mineralization of the agricultural soil treatments (Figure 4b).

Mineralization indices were highest for the fertilized CP soil with fertilizer and without residue (Trt 5) compared to the other five soil treatments (Figure 5). This was probably due to direct leaching of fertilizer N with the smallest total C evolu-

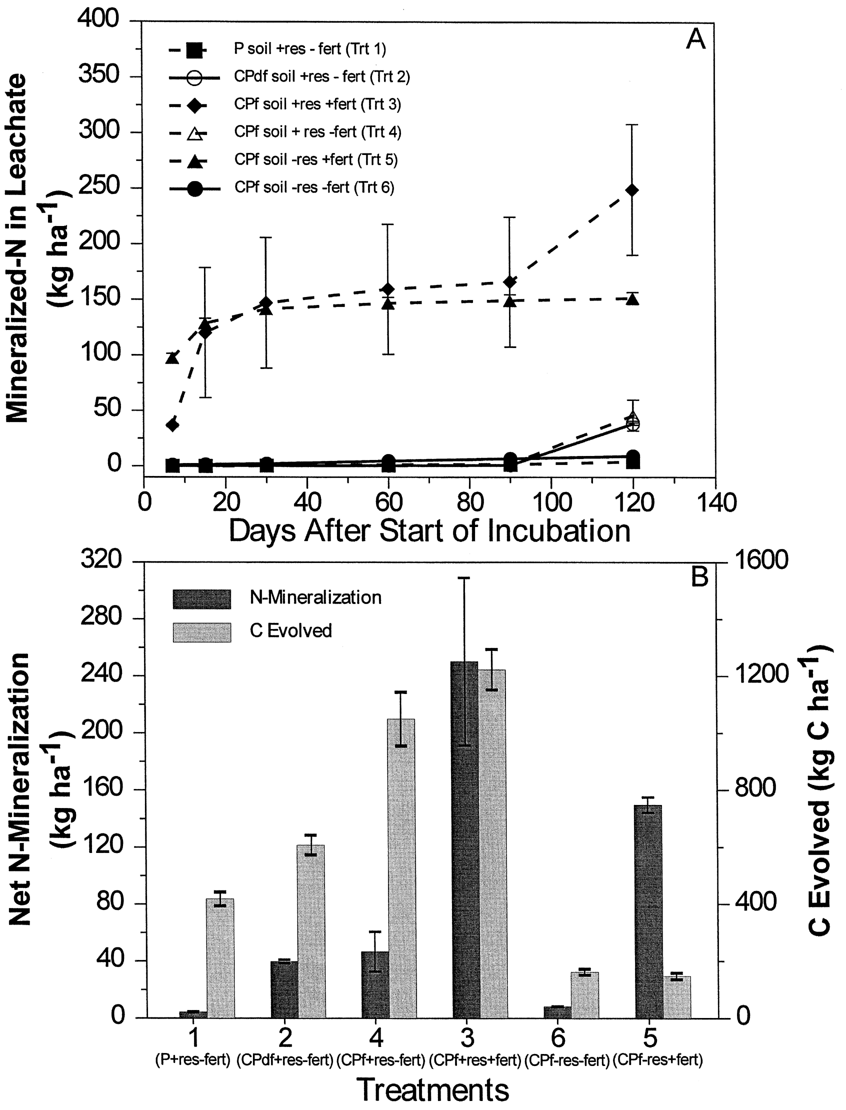

Figure 4. Cumulative inorganic N in leachate from laboratory incubation (A) and net N-mineralization and total C evolved for the 120 -d incubation period (B) for the six soil treatments consisting of either prairie (P), deficiently N-fertilized chisel-plowed (CPdf), or fertilized chisel-plowed (CPf) soil with (+) or without (-) fertilizer (fert) or residue (res) inputs. Standard error bars are provided for replicate samples. (Note: treatment labels are not in sequential order for Figure 4b to facilitate comparisons within natural groupings)

tion throughout the incubation period indicating minimal microbial activity (Figure 5a). The fertilized CP soil with fertilizer and residue (Trt 3) had a smaller mineralization index, but also had larger net N-mineralization and six-fold larger total C evolution compared to the fertilized CP soil with fertilizer and without residue (Trt 5). An exponentially decreasing mineralization index over time probably reflects the decreased rate of leaching of fertilizer N and limited microbial activity for Trt 5 (Figure 5b). Despite the slightly increasing mineralization index over time for the other five soil treatments, the amount of C substrate probably still limited microbial activity and/or N-mineralization processes in the other soil-fertilizer-residue treatment combinations as evidenced by the net N-mineralization and C-evolved responses for treatment pair 4 and 6 compared to treatment pair 3 and 5 (Figure 4b).

Discussion

Effects on inorganic soil N

Van Miegroet (1995) showed that available-soil-N-estimation methods, especially those that partition between ammonium- and nitrate-N, are generally affected by sample storage and handling that delays extraction after sample collection, even cold storage < 24 hr. This suggests that virtually all such data are affected to differing and unknown degrees. While this study's results were affected by pre-extraction sample handling, the results were consistent across the extended sampling period. The patterns of land-use effects on inorganic soil N, which were relatively consistent through time, lend support to the validity of the data.

Inorganic soil N was unaffected by tillage in the top 30 cm of deficiently N-fertilized NT and CP maize agroecosystems. The dominant inorganic N form varied by land use, where NH_4^+ was the dominant form in the prairie, while NO_3^- was the dominant form in the deficiently N-fertilized maize agroecosystems. This suggests that NH_4^+- and NO_3^--N were the sensitive inorganic N forms to monitor, because they were the less dominant form, in the managed agroecosystems and natural prairie, respectively.

Effects on direct and indirect net N-mineralization estimates

The effects of land use and management practices on net N-mineralization reported in the literature are highly variable (Table 1). Native prairie has been shown to have higher net N-mineralization than cultivated winter wheat (*Triticum aestivum* L.) (Ajwa et al. 1998) and similar net N-mineralization to reduced-tillage agroecosystems (Qian and Schoenau 1995), while uncultivated soil had higher net N-mineralization than cultivated soil (Hanlon et al. 1997). Net N-mineralization results reported in this study for several direct and indirect methods support the observations of previous studies using similar techniques. The CP maize agroecosystems typically had larger net N-mineralization than NT maize agroecosystems, while the optimally N-fertilized tillage treatments typically had greater net N-mineralization than deficiently N-fertilized tillage treatments (Figure 3), but large variability lim-

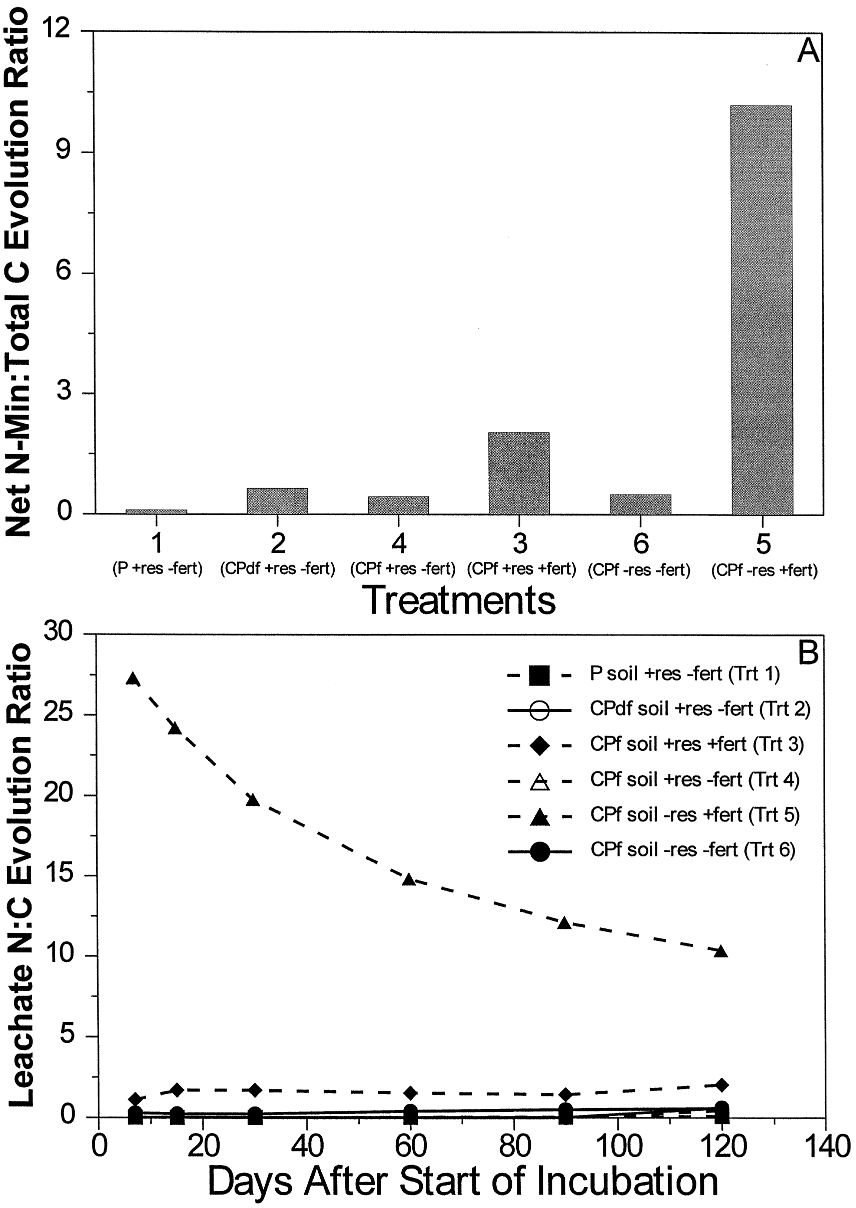

Figure 5. Nitrogen mineralization indices during laboratory incubation depicted as the ratio of net N-mineralization (kg N ha^{-1}) to total C respired for the entire incubation period (g C m^{-2}) (A) and as the ratio over time of cumulative mineralized N in the leachate solution (kg N ha^{-1}) to cumulative carbon respired (g C m^{-2}) (B) for the six soil treatments consisting of either prairie (P), deficiently N-fertilized chisel-plowed (CPdf), or optimally N-fertilized chisel-plowed (CPf) soil with (+) or without (–) fertilizer (fert) or residue (res) inputs. (Note: treatment labels are not in sequential order for Figure 5a to facilitate comparisons within natural groupings)

ited statistical significance (Brye 1999). Kolberg et al. (1999) reported that net N-mineralization increased with increasing fertilizer-N rates in dryland NT wheat-maize-fallow crop rotations in Colorado using a similar *in-situ* field technique. Net N-mineralization estimates in the maize agroecosystems were generally similar to net N-mineralization in many forested ecosystems, while net N-mineralization was generally smaller in the restored prairie than in forests (Table 1).

Inter-annual variability of net N-mineralization was, at times, as large as treatment differences among the agroecosystems. This may have been a result of climate variations from year to year, which have been shown to significantly affect the C budgets of the prairie and maize agroecosystems (Brye et al. 2002a (in press)). Growing season precipitation and mean daily air temperatures were similar for 1995, 1996, and 1998, but were considerably lower in 1997 (Brye et al. 2002b). Consequently, the annual estimate of net N-mineralization from 1997 may be less representative of typical net N-mineralization rates from years when moisture and temperature were closer normal, as was the case for 1995, 1996, and 1998.

The prairie's annual net N-mineralization estimated as the residual from the N budget was high in 1998 compared to the other years. Higher net N-mineralization in the prairie could have been the result of several interacting effects: i) an accumulation of inorganic soil N from spring to fall from decomposing roots following the prescribed prairie burn in April 1998 and ii) higher soil temperature and moisture early in the growing season as a result of the removal of the residue barrier, which may have stimulated microbial activity and mineralization.

The N-budget-residual and plant-N-uptake N-mineralization estimates account for processes in the entire soil profile (i.e., 0 to 1.2 m). Kessavalou and Walters (1999) concluded that some potential N supply to crops should be based on potential mineralizable-N below the surface horizon, especially in soils with incorporated residue. Though the majority of net N-mineralization occurs near the soil surface, as much as 20 to 45% of the total N-mineralization for the profile (Cassman and Munns 1980; Hadas et al. 1989) may occur below 60 cm (Jarvis et al. 1996). Scaling surface (i.e., 0–20 cm) net N-mineralization measurements to the soil profile (i.e., 0–120 cm) increased the annual net N-mineralization estimate. Between 50 and 60% of the net N-mineralization for the profile was estimated to have occurred below the top 20 cm.

Differences between the profile-scaled *in-situ* incubation estimates and the other two estimates of net N-mineralization may have been related to the profile-scaling factors, which were derived from measured C profiles and varied among land uses by roughly 30%. Scaling surface net N-mineralization estimates to encompass more of the root zone using soil C profiles is one attempt to account for sub-soil mineralization, but may not represent the best or most comprehensive method of doing so. Further work will be needed to define the most appropriate method for estimating net N-mineralization for the entire root zone.

Overall, mean annual net N-mineralization values for the three independent methods (Table 3) were within the range reported for previous studies using similar field techniques (Table 1). The mean net N-mineralization estimate for the three methods, calculated by pooling data across land uses and years, was 70 kg N ha^{-1} yr^{-1} with a standard deviation of 40 kg N ha^{-1} yr^{-1}, which was roughly 40% of the mean. This result suggests that the three independent net N-mineralization estimates agreed reasonably well when evaluated over multiple years, but the three methods were less consistent when comparing results within a single year.

Effects on net N-mineralization by laboratory incubation

Laboratory N-mineralization results supported the findings of Kolberg et al. (1999), who showed higher N-mineralization rates with increasing applied fertilizer-N, and provided evidence in support of a priming effect of N-mineralization processes in fertilized soils (Bingeman et al. 1953; Westerman and Kurtz 1973; Johnson 1992; Martin-Olmedo and Rees 1999). Although the priming effect has been suggested to be a procedural artifact (Jenkinson et al. 1985; Jarvis et al. 1996), net N-mineralization was greater for optimally N-fertilized (Trt 3 and 5, with and without residue, respectively) than deficiently N-fertilized soils (Trt 4 and 6, with and without residue, respectively), suggesting that if N was not added, little mineralization occurred (Figure 4b). Whether results of this study are confounded by a methodological artifact or not, the results represent a reasonable simulation of field conditions. Similar differences in soil mineral N content are likely to occur in the field.

Nitrogen mineralization rates appeared to have stabilized for all six soil treatments by 90 d into the incubation (Figure 4a). This result was similar to that of Bonde and Rosswall (1987) who reported that the majority of the mineralized-N is $CaCl_2$-leached early and decreases into the incubation. However, inorganic N in the leachate of all three residue-amended agroecosystem soil treatments (i.e., Trt 2, 3, and 4) increased between 90 and 120 d into the incubation. This result suggested that a flush of mineralized-N occurred, possibly as a result of microorganism mortality and turnover releasing a very labile C source, which stimulated N-mineralization by the remaining or new microbial population. Consequently, the residue-amended fertilized soil (Trt 3) yielded a net N-mineralization (i.e., 250 kg N ha^{-1}) that was greater than the amount of fertilizer-N applied (i.e., 190 kg N ha^{-1}), meaning that roughly 60 kg N ha^{-1} was mineralized from native soil organic matter (i.e., a “priming” effect).

Similarly, Rasmussen et al. (1998) found that previous N fertilization affected N-mineralization in laboratory incubations from 7 to 49 d long. However, mineralized-N in the leachate and net N-mineralization did not differ significantly (p-value > 0.05) among the deficiently N-fertilized CP soil with residue and without fertilizer (Trt 2) and the fertilized CP soil with residue and without fertilizer (Trt 4) treatments, suggesting that past soil fertilization history did not affect N-mineralization processes in the Plano silt loam soil during laboratory incubations (Figure 4a and 4b).

Though past soil fertilization history did not seem to affect net N-mineralization, total C evolved during the incubation was higher for the fertilized CP soil with residue and without fertilizer (Trt 4) compared to the deficiently N-fertilized CP soil with residue and without fertilizer (Trt 2). This suggests that past soil fertilization affects soil CO_2 flux (Figure 4b). Similarly, net N-mineralization and total C evolved were greater for the fertilized CP soil with residue and without fertilizer (Trt 4) compared to the prairie soil with residue (Trt 1) (Figure 4b), further illustrating the dependence of microbial-mediated processes on soil fertility.

Effects on C and N dynamics

Crop residues returned to the soil surface or incorporated by tillage following harvest become a source of labile C for the next growing season. Microbe-mediated processes, such as N-mineralization, become C-limited if a labile C source is not replenished with residue (i.e., Trt 5 and 6). Laboratory incubation results suggested that net N-mineralization was roughly 40% less in agricultural soils without incorporated residue compared to agricultural soils with incorporated residue (Figure 4b). This incorporated-residue effect on N-mineralization in agricultural soils has been observed before in field and laboratory studies (Jarvis et al. 1996). However, a N-amendment effect was not observed with soil CO_2 flux (Figure 4b), though the mean estimate of total C evolved from the fertilized CP soil with and without fertilizer treatments (Trt 3 and 4, respectively) during the laboratory incubation (i.e., 3408 kg C ha^{-1} yr^{-1}) compared reasonably well to annual estimates obtained from field measurements on the same soil treatments (i.e., 5215 kg C ha^{-1} yr^{-1}) (Wagai et al. 1998).

Cultivated agriculture has been shown to affect the seasonal variation of inorganic N and labile C (DeLuca and Keeney 1994). The freezing and thawing that surface soils experience over the winter significantly increase the soil mineral N content and N-mineralization rates following spring thaw (DeLuca et al. 1992). Inorganic N and labile C are generally more depleted following harvest than before planting in agricultural soils (Bonde and Rosswall 1987). Consequently, the timing of sample collection (i.e., fall versus spring) may introduce unforeseen variation, which could yield erroneous interpretation of laboratory results relative to N and C dynamics and how much N-mineralization can be expected to contribute to a crops N requirement for optimal production. Better understanding of the mechanisms influencing microbial processes, such as N-mineralization, may be gained from using soils depleted of N and C (i.e., fall-sampled soils) versus soils already enriched with readily available N and labile C (i.e., spring-sampled soils) due to the effects of freeze-thaw phenomena on surface soils.

Summary and conclusions

Nitrogen mineralization is a spatially variable and difficult component of the N cycle to quantify accurately under field conditions. Obtaining reliable estimates of net N-mineralization during the growing season will improve commercial fertilizer recommendations and help minimize undesirable, environmental N enrichment.

Land use and management practices (i.e., restored prairie, deficiently and optimally N-fertilized, NT and CP maize monoculture) significantly affected direct and indirect annual net N-mineralization estimates. Net N-mineralization was consistently smaller in the restored prairie than in the maize agroecosystems and typically larger in the CP than in the NT maize agroecosystems. Residue-amended soil had higher net N-mineralization than soil with no added residue. *In-situ* field measurements suggested that net N-mineralization was higher in optimally N-fertilized than deficiently N-fertilized soils, but the variability was generally too high for the field measurements in the maize agroecosystems to yield a statistically significant inference.

Though not similar in magnitude, patterns of land use, tillage, and fertilizer-N-rate effects on annual net N-mineralization were relatively consistent across several indirect estimation methods and over a multi-year period. This type of comprehensive field evaluation of net N-mineralization for several typical agroecosystems establishes a range of expected values for assessing method-to-method, inter-annual, or treatment-to-treatment variations.

Net N-mineralization by laboratory incubation and periodic leaching of fall-sampled soils supported field measurements by showing a significant difference in net N-mineralization with and without added fertilizer-N. Withholding residue inputs to agricultural soils significantly decreased net N-mineralization and total C evolution compared to residue-amended agricultural soils; likewise, withholding fertilizer inputs from agricultural soils significantly decreased net N-mineralization compared to fertilized soils. Consequently, both fertilizer-derived mineral N and residue-derived labile C were apparently co-limiting factors influencing N-mineralization in agricultural soils.

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