

Nitrogen Immobilization and Mineralization Kinetics of Cattle, Hog, and Turkey Manure Applied to Soil

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Nitrogen mineralization and immobilization following manure application are critical processes influencing plant N supply and offsite N losses. We conducted laboratory experiments to examine the influence of these two processes in addition to N oxide gas production on N availability for 180 d following manure addition. A Tara loam (fine-silty, mixed, superactive, frigid Aquic Hapludoll) and a Webster clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquoll) amended with liquid dairy (*Bos taurus*) manure (LD) were incubated at 25 and 10°C, while Tara soil amended with solid dairy manure (SD) and turkey (*Meleagris gallopavo*) manure (T), and Webster soil amended with liquid hog (*Sus scrofa*) manure (H), were incubated at 25°C. Maximum net N immobilization was 14 and 40% of the initial NH_4^+ concentration in H and LD, respectively, and persisted for 35 to 180 d. In LD-, H-, and T-amended soils, net manure N mineralization was not apparent, and there was good agreement between initial NH_4^+ content and available inorganic N from the manure. These data suggest that, for these manure types, estimates of first-season available N would be improved by measuring manure NH_4^+ . In contrast, in soil amended with SD, which had the lowest initial NH_4^+ content, 22% of organic N was mineralized. Gaseous N losses were <1% of the added N in all treatments. The previously developed model NCSOIL was used to predict plant N availability and NO_3^- leaching potential with various manure incorporation dates. Under climate conditions typical of the Upper Midwest, no clear advantage of late fall compared with spring incorporation of manure with regard to N availability could be shown, but NO_3^- leaching potential seemed high with early fall incorporation.

Abbreviations: H, liquid hog manure; LD, liquid dairy manure; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SD, solid dairy manure; T, turkey manure.

Manure application to cropland supplies essential nutrients (Sims and Wolf, 1994), increases soil organic matter (Habteselassie et al., 2006), and reduces erosion (Gessel et al., 2004). Apart from these beneficial effects, high N and P inputs due to manure applications may also pose environmental risks (Gupta et al., 2004). Therefore, N and P nutrient analyses and crediting before farmland application are widely advocated or, in environmentally sensitive areas, mandated by law (Schmitt et al., 1999). Accurate estimates of plant-available N and P are needed to maximize the benefit to the crop and to minimize undesirable losses of these nutrients.

Manures vary widely in quality as soil amendments, depending on the animal production systems and the manure treatment, storage, and application methods. The varied components and properties of manure affect N mineralization, N

immobilization, and the denitrification potential of the soil to which they are applied. Declines in inorganic N occurring immediately (days to weeks) after manure application to soil have been attributed to NH_3 volatilization, denitrification, or microbial immobilization. Nitrogen losses as high as 39% of the manure N via denitrification have been reported from soil microcosms (Calderón et al., 2005). In other experiments, N_2O emissions from manure-amended soil amounted to <1% of the manure N (Paul et al., 1993). Estimates of net N immobilization range from about 20% of total N after cattle slurry (Sørensen and Jensen, 1995) to >70% for anaerobically treated pig slurry applications (Kirchmann, 1991). Net N immobilization after manure applications, i.e., inorganic N levels lower than the initial levels, lasted from 20 d (Kirchmann and Lundvall, 1993) to >20 wk (Sørensen and Amato, 2002). The magnitude of net N immobilization and the rates of inorganic N production are critical knowledge for optimizing plant N supply by choosing an appropriate manure application date.

The C/N and NH_4^+ /organic N ratios of applied manures used as soil amendments have been used as potential predictors of plant-available N because these properties influence N immobilization and mineralization (Beauchamp and Paul, 1989). The NH_4^+ in manure is often assumed to be the fraction of N available for crop uptake in the year of application (Olesen et al., 2004). Sometimes recommended manure application rates are based solely on the total N (or total P) content of a given manure (Schmitt and Rehm, 1998). Computer

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programs that calculate manure application rates generally assume that 100% of the $\text{NH}_4^+\text{-N}$ and some percentage (0–50%) of the organic N become available for plant uptake during the growing season immediately following application (Thompson et al., 1997). There are few data available to support these assumptions, however, especially under specific manure, soil, and climate conditions.

Estimates of net N mineralization and inorganic N availability can be made by incubating manure-amended soil under controlled conditions. Studies of this kind have contributed to elucidating the influence of storage method (Kirchmann, 1991), soil texture (Sørensen and Jensen, 1995), and manure composition (Van Kessel et al., 2000) on the N mineralization-immobilization dynamics of manure-amended soil. Research is needed to more reliably relate manure properties that can be easily measured before application to actual N availability, and to explore simulation modeling as a decision support tool for manure management.

The overall goal of the present study was to estimate N availability to plants during the first growing season after application of manures that differed in type, C/N ratios, and the proportions of inorganic and organic N contents, namely liquid dairy, solid dairy, turkey, and liquid hog manures. The specific objectives were to (i) understand the influences of microbial N immobilization, manure N mineralization, and gaseous N production on manure N availability and (ii) to predict N availability in the field by using a mechanistic model that simulates C and N turnover in soils (NCSOIL).

MATERIALS AND METHODS

Manure and Soil

Laboratory incubations were performed with four types of manure that are customarily applied on farmers' fields in Minnesota. Liquid dairy and hog manure were collected from storage pits underneath animal confinement buildings. Solid dairy manure was obtained from an open shed with straw bedding accessible to cattle in outdoor lots. Turkey manure was taken from a large outdoor stockpile that included sawdust and wood chips. The SD and T were approximately 2 mo old at the time of collection, whereas LD and H had accumulated in the pits for several months. All the manures were stored at 4°C for another 3 mo, the liquid manures in air-tight jars and the solid manures in closed plastic containers.

One of the soils was from the surface layer (0–30 cm) of a Tara loam from a field under wheat (*Triticum aestivum* L.) in Morris, MN (45°35' N, 95°55' W), with 24 g C kg⁻¹ soil, 2.1 g N kg⁻¹ soil, and a pH of 6.5. The soil texture was 270 g kg⁻¹ sand, 470 g kg⁻¹ silt, and 260 g kg⁻¹ clay. The second soil was collected in Waseca, MN (44°5' N, 93°31' W), from the surface layer of a Webster clay loam that had

not received any N inputs since 1986 and had been under continuous corn (*Zea mays* L.) since 1972. This soil had 24 g C kg⁻¹ soil, 1.9 g N kg⁻¹ soil, and a pH of 5.1. The soil texture was comprised of 420 g kg⁻¹ sand, 290 g kg⁻¹ silt, and 290 g kg⁻¹ clay. The soils were air dried, sieved (4 mm), and stored at 4°C for 3 mo.

Selected manure properties are shown in Table 1. Total N and P of each manure type were determined by the modified Kjeldahl method using H_2SO_4 and H_2O_2 , followed by colorimetric analysis of the extracts (Forster, 1995; Murphy and Riley, 1962). Total C was measured by a C and N analyzer (Carlo Erba, Milan, Italy) using lyophilized samples. Ammonium N in the manures was measured colorimetrically in 2 mol L⁻¹ KCl-diluted, filtered (0.45- μm) manure subsamples (Forster, 1995). Manure organic N (N_{org}) was calculated as the difference between manure total N and $\text{NH}_4^+\text{-N}$, since NO_3^- concentrations were below detection limits in all four manure types.

Soil Incubations

For each of 11 treatments, 24 replicate microcosms were prepared. For the 25°C incubation, the treatments were LD, SD, T, and control in the Tara soil and LD, H, and control in the Webster soil (Table 1). For the 10°C incubation, the treatments were LD and control for each soil type. Shortly before the start of the incubations, the manures were diluted with deionized water and then homogenized with dry ice in a blender (Calderón et al., 2004). Microcosms were prepared by adding 5 to 8 mL of the homogenized manure slurry to 60 g dry weight soil in 120-mL plastic cups. For each microcosm, approximately 20 g of soil was first placed into the cup. One aliquot of manure slurry was then applied with a pipette that was slowly discharged as the pipette tip was moved across this layer of soil. Another 20 g of soil was placed on top of the bottom layer, and a second aliquot of manure was applied in the same manner. Finally, the remaining soil was placed on top of the two layers. Deionized H_2O was added to bring each microcosm to a water content of 60% water holding capacity (WHC). By using this value of WHC, we achieved similar values of water-filled pore space in the two soils. No mixing took place, as this would have led to nonuniform bulk density. The careful dispensing of the slurry and the fact that all manures were applied as a liquid ensured uniform distribution. Each microcosm received 154 mg N kg⁻¹ soil as manure (~ 300 kg N ha⁻¹). The control microcosms did not receive manure amendments. For the 25°C incubation, the LD and control microcosms received 2 and 1 mg N kg⁻¹ as 98 atom% ¹⁵N-enriched $(\text{NH}_4)_2\text{SO}_4$, respectively. Each plastic cup was placed into a 1-L glass jar sealed with a lid equipped with butyl rubber septa and incubated at either 25 or 10°C in the dark. Deionized H_2O (2 mL) was placed in the bottom of each jar to maintain humidity. The jars were aerated weekly during the first 4 wk and every 2 wk thereafter, and microcosm moisture was replenished at these occasions if necessary. Soil pH was measured in a supernatant of soil slurries of 1 mol L⁻¹ KCl

Table 1. Total N (N_{tot}), $\text{NH}_4^+\text{-N}$, total C, and total P on a dry manure basis, N_{tot} , $\text{NH}_4^+\text{-N}$ and total C on a soil dry weight basis, as well as the C/total N ratio ($\text{C}/\text{N}_{\text{tot}}$) of the manures applied to Tara or Webster soil and incubated at 25 or 10°C.

Manure	Soil	Incubation temperature °C	N_{tot}	g kg^{-1} manure			$\text{C}/\text{N}_{\text{tot}}$	N_{tot}	mg kg^{-1} soil	
				$\text{NH}_4^+\text{-N}^\dagger$	C	P			$\text{NH}_4^+\text{-N}^\dagger$	C
Liquid dairy	Tara, Webster	25, 10	38	16	427	3	11.3	154	64	1740
Solid dairy	Tara	25	25	1	429	10	17.2	154	5	2640
Turkey	Tara	25	45	16	354	40	7.8	154	50	1200
Liquid hog	Webster	25	102	69	381	17	3.7	154	104	580
Control	Tara, Webster	25, 10	–	–	–	–	–	–	–	–

† Concentrations of NO_3^- were below detection limits.

and an equal mass of soil (Venterea and Rolston, 2000). The pH was measured before and 3 h after adding the manure, as well as on Day 70 of the incubation.

Destructive sampling of the microcosms (three replicates per treatment) was performed 0, 8, 21, 35, 50, 70, 133, and 180 d after the start of the incubation. At the first sampling (0), the entire soil sample of one cup was extracted with 2 mol L⁻¹ KCl at a liquid/soil mass ratio of 5. At the later samplings, a subsample of at least 20 g dry weight soil per microcosm was used for this analysis. Nitrate-N and NH₄⁺-N were measured colorimetrically (Doane and Horwath, 2003; Forster, 1995). Microbial biomass N (MBN) and microbial biomass C (MBC) were determined 8, 21, 35, 50, 70, 133, and 180 d after the start of the incubation using the 1-d chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Fumigated and unfumigated subsamples of 20 g dry weight soil of the LD and control treatments incubated at 25°C were extracted with 0.5 mol L⁻¹ K₂SO₄ at a liquid/soil mass ratio of 5. To estimate MBC, dissolved organic C (DOC) in diluted extracts was measured by an automatic analyzer (Phoenix 8000, Tekmar-Dohrman, Mason, OH). Microbial biomass N was determined after a modified Kjeldahl digestion (Wyland et al., 1994) of the extracts. Diffusion techniques were used to prepare the 2 mol L⁻¹ KCl and the digested MBN extracts (Stark and Hart, 1996). The three samples per treatment and date were pooled for the diffusions if the N concentration of each sample did not deviate >5% from the mean of these three samples; if the deviation from the mean of one of the samples was >5%, each sample of extract was diffused separately. Isotope ratios were determined by a Carlo Erba NA 1500 Elemental Analyzer with a Fison's Optima mass spectrometer.

Soil respiration was measured, using NaOH traps placed in the jars, in the three replicates that were subsequently destructively sampled. The NaOH was titrated with 0.5 mol L⁻¹ HCl. Additionally, discrete gas samples (9 mL) were taken from three replicate microcosms before every aeration. The samples were stored in evacuated 9-mL vials and analyzed for N₂O using a headspace autosampler (Tekmar Model 7000, Teledyne Tekmar, Mason, OH) connected to a gas chromatograph (Hewlett-Packard Model 5890) with electron capture detection. To a separate set of microcosms, 10 mL of C₂H₂ was added (Ryden et al., 1979) 6 d after the start of the incubation. Gas samples were taken before the C₂H₂ addition and 24 h later to obtain an estimate of total denitrification when C and NO₃⁻ availability and nitrification activity were assumed to be high.

Instantaneous rates of NO production of the soil samples were measured by a flow-through system connected to a NO_x analyzer (Models LMA-3 and LMA-3D, Unisearch, Inc., Concord, ON, Canada) (Venterea and Rolston, 2000).

Data Analysis and Statistics

Net mineralized inorganic N (N_m) was calculated as

$$N_m = (N_i)_t - (N_i)_{t=0} \quad [1]$$

where (N_i)_t is the inorganic N concentration on Day *t* and (N_i)_{t=0} is the inorganic N concentration at the start of the incubation for each treatment.

Microbial biomass N was calculated as

$$MBN = \left(NH_4^+ - N_{\text{fumigated}} - NH_4^+ - N_{\text{unfumigated}} \right) / 0.54 \quad [2]$$

where NH₄⁺-N_{fumigated} is the NH₄⁺-N in the fumigated sample and NH₄⁺-N_{unfumigated} is the NH₄⁺-N in the unfumigated sample (Shen et al., 1984; Brookes et al., 1985).

Microbial biomass C was calculated as

$$MBC = \left(DOC_{\text{fumigated}} - DOC_{\text{unfumigated}} \right) 2.64 \quad [3]$$

where DOC_{fumigated} is the DOC in the fumigated sample and DOC_{unfumigated} is the DOC in the unfumigated sample (Vance et al., 1987).

Gross immobilization of NH₄⁺ (I_{NH4}, mg N kg⁻¹) was calculated as

$$I_{NH_4} = MBN \left(A_{MBN} / A_{NH_4} \right) \quad [4]$$

where A_{MBN} is the atom% excess ¹⁵N of MBN on Day 8 and A_{NH4} is the atom% excess ¹⁵N of the soil NH₄⁺ at *t* = 0.

The data were analyzed as a two-way ANOVA, using the generalized linear models procedure (SAS Institute, 2006), with treatment and sampling date as factors. Treatment × date interaction effects were assessed using Tukey's multiple comparison test.

Simulation Modeling

The C and N transformations in the control and manure-amended soils were simulated using the model NCSOIL, which computes the changes in organic forms of C and N, inorganic N concentrations, and CO₂ release as a result of residue decomposition, mineralization, immobilization, and nitrification (Molina et al., 1983). The model assumes first-order kinetics of organic matter decomposition, as proposed by Stanford and Smith (1972). Three organic matter pools of the soil and two or three pools of the added manure were considered: Microbial biomass (Pool I) with a labile and resistant component, soil organic matter excluding biomass (Pool II), and two or three pools representing different organic fractions of manure (Pools III, IV, and V). Each pool was defined by its potentially mineralizable C (C_o), C/N ratio, decomposition rate constant, and microbial C use efficiency factor. The MBC and MBN were measured inputs. The C/N ratio of Pool II was assumed to be (C_{tot} - MBC)/(N_{tot} - MBN - N_i). The previously tested decomposition rate constants of Pool I labile, Pool I resistant, and Pool II were assumed to be 0.33, 0.04, and 0.006 d⁻¹ (Hadas et al., 1987; Molina et al., 1983), respectively. Thus, in the model, the labile and resistant fractions of the microbial biomass, which turns over rapidly, have half-lives of 2 and 17 d. The decay rate constant of Pool II is the value that was found to be very similar under optimal conditions for most of 39 widely differing soils studied by Stanford and Smith (1972). The microbial C use efficiency factors for Pool I were set at 0.6 (Hadas et al., 1987; Molina et al., 1983) and for Pool II at 0.4. Hadas et al. (1992) reported microbial C use efficiency factors for Pool II ranging from 0.37 to 0.42 after optimization of data simulated by different versions of NCSOIL. The C_o of Pool II was estimated by optimization of the model, i.e., by comparing model output to the experimental data of CO₂ evolution and inorganic N concentration of the control soils at the eight time points. The organic fraction of the manures was treated in the model as consisting of two or three components: a rapidly decomposing (Pool III) and one or two slowly decomposing (Pools IV and V) organic matter pools. The decomposition rate constants were assumed to be 0.9, 0.006, and 0.001 d⁻¹, and the microbial C use efficiency factors were set at 0.6, 0.4, and

0.4, respectively. The parameter values for Pool III were based on data obtained from glucose-amended soils (Nicolardot et al., 1994b). The decay rate of Pool IV was based on the decomposition rates of straw in soil (Paul and Juma, 1981) because dairy cattle rations often include silage and hay made from grasses of varied digestibility (Van Kessel et al., 2000), and straw was used as bedding for the dairy cattle. For Pool V, a median value was chosen based on the decomposition rate of biosolids [0.0015 d^{-1} (Gilmour et al., 2003)] and of composted cattle manure [0.000635 d^{-1} (Hadas and Portnoy, 1997)]. The microbial C use efficiency factor for Pools IV and V was based on the C utilization efficiency reported for biosolids (Gilmour et al., 2003), cellulose, and hemicellulose (Voroney et al., 1981). The sums of the C and N contents of these two or three pools were equal to the measured total C and N content of the organic fraction of the manure; however, the size and C/N ratio of each pool was derived by optimization of the model, using the experimental data and the above parameter values from the literature.

For the simulations of the control and LD-amended soils, the temperature was varied according to the experimental conditions, but all other parameters were left unchanged. In NCSOIL, the temperature factor, applied to microbial activity, varies in linear fashion between 0 (0°C) and 1 (30°C). The model simulated NO_3^- concentrations after fall (30 September or 31 October) and spring (15 April or 5 May) incorporation of LD and SD in Tara soil, using 37-yr average soil temper-

atures at the 10-cm depth at the Southern Research and Outreach Center of the University of Minnesota, Waseca.

RESULTS AND DISCUSSION

Nitrogen Mineralization and Immobilization

The manures, which were applied to the soil at equal rates in terms of total N, differed in C content and in the proportion of N in the form of NH_4^+ vs. N_{org} , as well as in their total P content (Table 1). In the Tara soil, virtually all NH_4^+ disappeared within 8 d in the LD 25°C treatment and within 21 d in all the remaining treatments (Fig. 1). In the Webster soil, most NH_4^+ disappeared within 21 d in the LD treatment, but sizable NH_4^+ pools persisted longer than 21 d in LD 10°C (70 d) and H. The relatively rapid disappearance of NH_4^+ during the first 1 to 3 wk of incubation was accompanied by increases in NO_3^- . After 180 d, >99 and >97% of inorganic N present in the manure treatments and controls, respectively, was in the form of NO_3^- . The pH was not significantly affected by the treatments at any time (data not shown).

Differences in NO_3^- availability in the manure-amended soils at the end of the 180-d incubations mirrored the differences in initial NH_4^+ in three of the four manure treatments (LD, T, and H). The greatest amount of available inorganic N within 180 d of application was from the manure with the

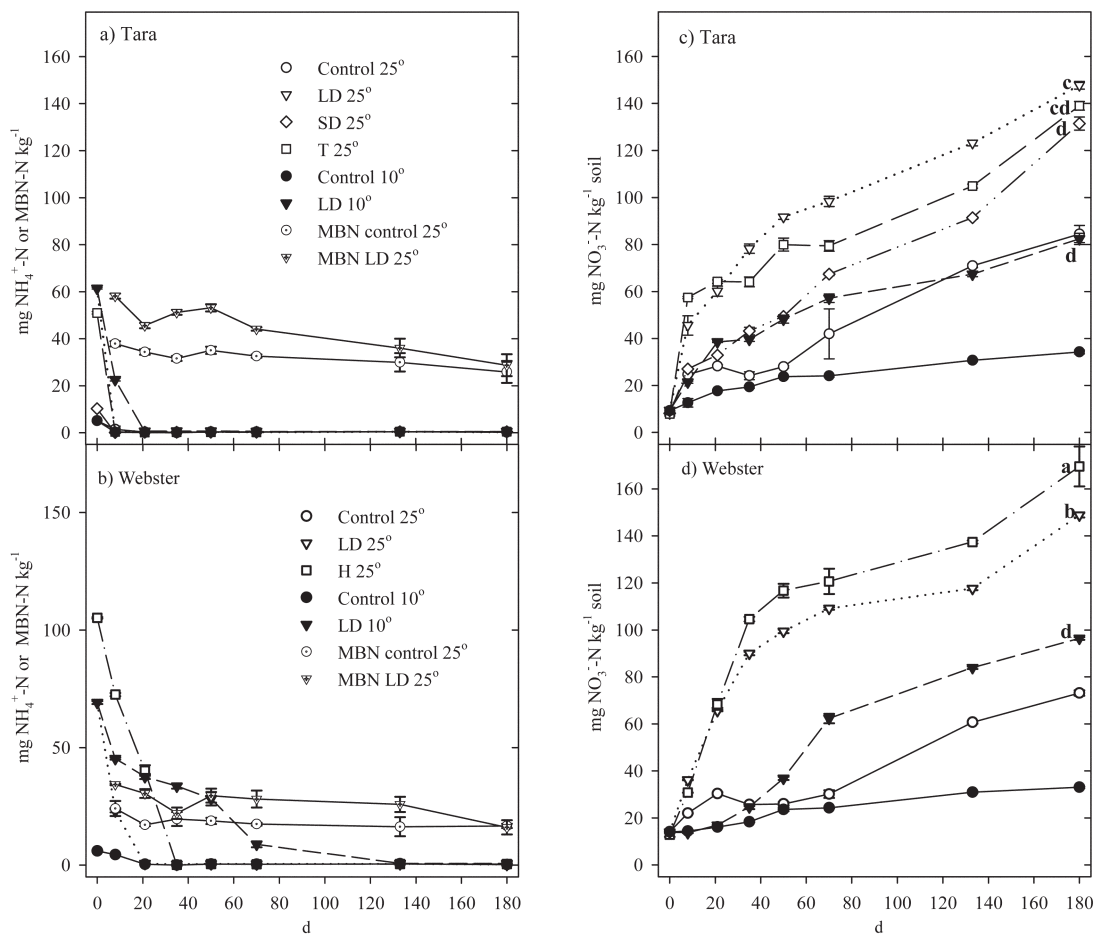


Fig. 1. Ammonium concentrations in (a) Tara and (b) Webster soils amended with liquid dairy manure (LD), solid dairy manure (SD), turkey manure (T) and hog manure (H), and NO_3^- concentrations in (c) Tara and (d) Webster soils amended with LD, SD, T, and H during incubation at 25 or 10°C . In (c) and (d), means (Day 180) designated with the same letters are not significantly different with regard to NO_3^- accumulated above controls. Concentrations of microbial biomass N (MBN) of control and LD-amended soils are also shown in (a) and (b). Standard errors are shown as line bars.

greatest NH_4^+ content (H; Fig. 1). At 25°C, NO_3^- availability above the control was higher in LD than SD, which had the lowest initial NH_4^+ concentration.

In the LD-amended soils, inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) declined in the first week of the incubation by 23.8 to 26.7 mg N kg^{-1} at both temperatures (Fig. 2). During the first week of the incubation, MBN immobilization, based on ^{15}N tracer data measured in the LD 25°C treatments, was 20.7 and 20.3 mg N kg^{-1} for Tara and Webster soils, respectively. By comparison, microbial gross N immobilization in the control soils was 0.8 (Tara) and 2.9 mg N kg^{-1} (Webster). Temporary decreases in inorganic N concentrations after application of manure have often been attributed to microbial immobilization (Flowers and Arnold, 1983; Kirchmann and Lundvall, 1993; Sørensen and Jensen, 1995), and MBN has been shown to increase as inorganic N in the soil decreased (Calderón et al., 2005; Sørensen and Amato, 2002). The uptake of ^{15}N -labeled NH_4^+ by the microbial biomass in the present study is direct evidence that the decline in inorganic N in the LD treatments was mainly due to microbial immobilization. The MBN concentration data corroborated the results of microbial gross N immobilization based on uptake of the ^{15}N -labeled NH_4^+ . For

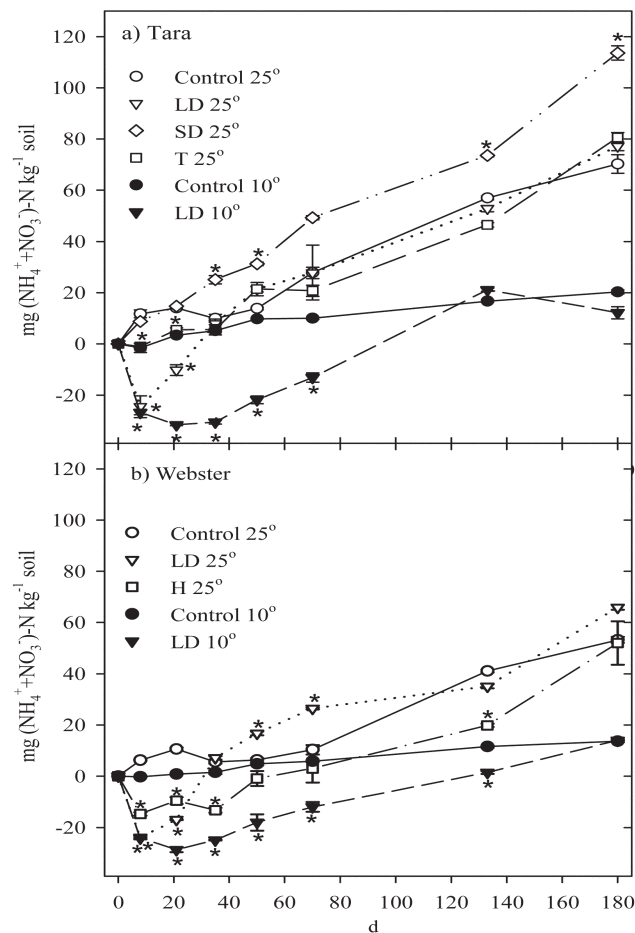


Fig. 2. Net inorganic N mineralized since the start of incubation at 25 or 10°C in (a) Tara or (b) Webster soil. The treatments were: control without N addition, liquid dairy manure (LD), solid dairy manure (SD), turkey manure (T), and hog manure (H) amendment. *Means of manure treatments are significantly different from controls within the same day at $P = 0.05$ ($n = 3$). Standard errors are shown as line bars.

both soil types, the MBN concentration on Day 8 was significantly higher in the LD-amended than the control soils, the mean differences being 20.3 and 10.1 mg N kg^{-1} soil for Tara and Webster, respectively (Fig. 1). Figure 3 shows the fate of the $\text{NH}_4^+ -^{15}\text{N}$. Mean overall recoveries of ^{15}N were 81 (± 5), 85 (± 5), 67 (± 9), and 57 (± 7)% in LD Tara, LD Webster, control Tara, and control Webster, respectively. The variability of overall ^{15}N recovery was unlikely to have affected the results of gross microbial $\text{NH}_4^+ -^{15}\text{N}$ immobilization. In the calculation of gross microbial NH_4^+ immobilization, N isotope ratios of MBN and NH_4^+ , and MBN mass were considered (Eq. [4]). On Day 0, the entire sample of soil of each microcosm was extracted with KCl. Therefore, errors due to insufficient soil mixing could not have influenced the isotope ratios of soil NH_4^+ , and incomplete extraction would not have affected the N isotope ratios either. Furthermore, the trends in MBN concentration and MBN- ^{15}N recovery data are similar, which indicates that MBN isotope ratios were consistent.

Microbial gross N immobilization was 32% of the NH_4^+ initially present in the manure, or 13% of the manure's total N, whereas gaseous N losses were <1% of total N (see below). It is probable that the significantly lower N_m concentrations in the H and T treatments during the first 21 to 35 d of the incubation compared with the control were also the result of microbial N immobilization. Although inorganic N concentrations did not decline in the SD-amended soil, N immobilization may have been occurring concurrently with N mineralization.

In the LD treatments, N immobilization was equally rapid at both incubation temperatures. The high microbial N demand suggests the existence of a labile C source such as volatile fatty acids (VFA), which have been measured in manure slurries (Patni and Jui, 1985; Paul and Beauchamp, 1989). Concentrations of VFA in soil after application of cattle slurry decreased from >1000 mg C kg^{-1} to almost nil within <4 d (Paul and Beauchamp, 1989), and Kirchmann and Lundvall (1993) showed a correlation between the initial VFA in dairy and pig slurries and inorganic N disappearance. Volatile fatty acids are intermediate products of anaerobic biodegradation that may have been present in our LD and H slurries due to

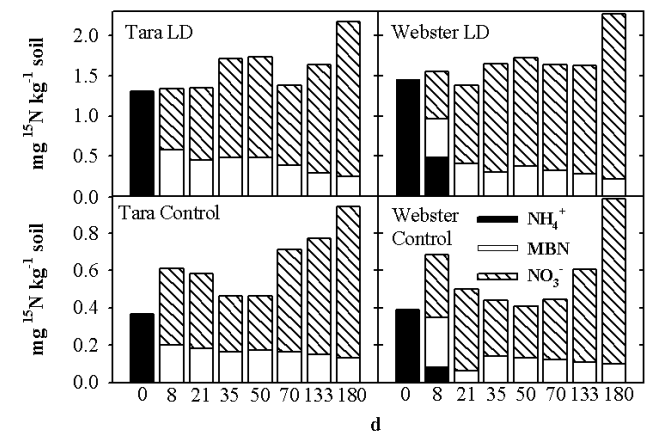


Fig. 3. Recovery of ^{15}N in NH_4^+ , microbial biomass N (MBN), and NO_3^- pools in Tara and Webster soils amended with liquid dairy manure (LD) or without amendment (control), incubated at 25°C for 180 d. The manure-amended and control soils received 2 or 1 mg 98% ^{15}N enriched $(\text{NH}_4)_2\text{SO}_4\text{-N kg}^{-1}$ soil, respectively, at the start of the incubations.

storage in pits underneath confinement buildings and in the closed jars. It is interesting that the magnitude of net N immobilization was similar at both temperatures although CO₂ emissions during the first 8 d were relatively lower at 10 than at 25°C (Fig. 4 and 5). A possible explanation of these differences in CO₂ emission and similarities of net N immobilization under the two temperature regimes could be that microorganisms used mainly labile C for biomass production. Devèvre and Horwath (2000) found that biomass production during straw decomposition was similar at 5 and 25°C, but at the higher temperature, microorganisms decomposed recalcitrant fractions of C, such as cellulose and lignin, in addition to labile C.

Inorganic N immobilization was followed by N mineralization. The amount of N mineralized since the start of the incubation (N_m), i.e., net mineralization of organic N, is shown in Fig. 2. At 25°C, by Day 35 (Tara) and Day 50 (Webster), N_m in the LD-amended and control soils were at similar levels until Day 180, with the exception of two intermediate sampling dates in the Webster soil. In the H-amended soil, N_m was lower than in the control during most of the time of the incubation. After 180 d, however, N_m was similar in H-amended

and control soils. In the T-amended soil, N_m was similar to the control soil except for the first two sampling dates.

During the 180-d incubation, no net mineralization of manure organic N (N_{org}) was apparent in the LD, T, and H treatments since N_m at each sampling date was either lower than or did not differ from N_m in unamended soils. Therefore, in these three treatments, the amount of N potentially available from manure in the year of the application could have been predicted based on the NH₄⁺ content of the manure. Such an apparent lack of net N mineralization after application of manure slurries has been observed in other extended incubation studies. During a 175-d incubation of soils amended with fresh pig slurry, there was no evidence of an increase in N mineralization arising from either the initially immobilized inorganic N or the manure organic N (Flowers and Arnold, 1983). In contrast, in soil amended with anaerobically stored pig or cattle slurry, net N mineralization increased following a few days of net N immobilization, and after another 20 d, net N mineralization was similar to the control soil for the remainder of the 70-d incubation (Kirchmann and Lundvall, 1993). In the present study, similar N dynamics as in the latter

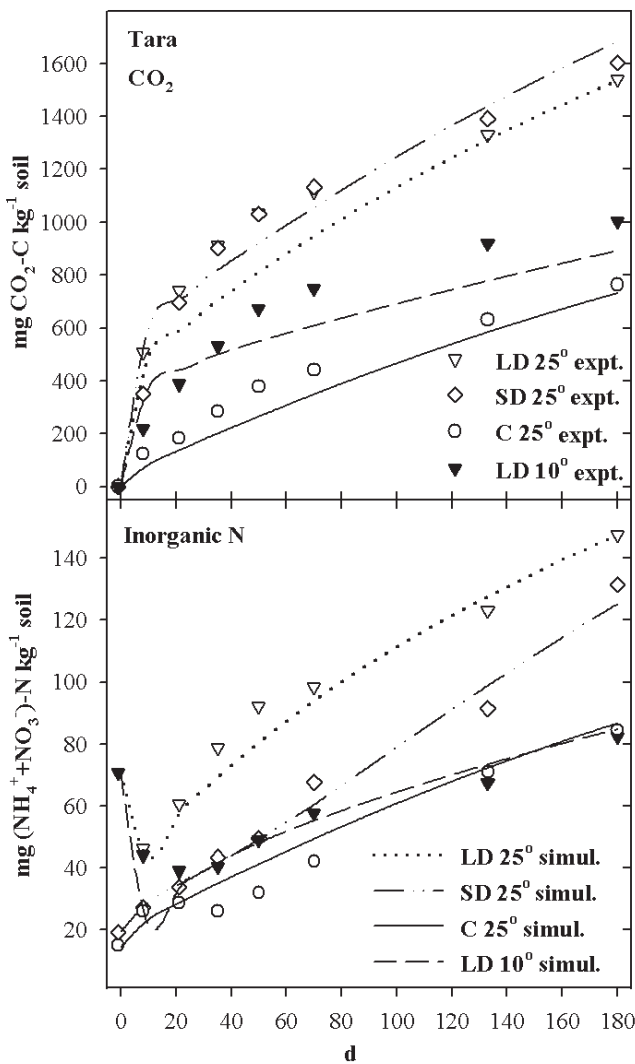


Fig. 4. Experimental (symbols) and simulated (lines) values of cumulative CO₂ and inorganic N of Tara soil amended with liquid dairy manure (LD) and solid dairy manure (SD) and unamended control (C) during incubation at 25 or 10°C.

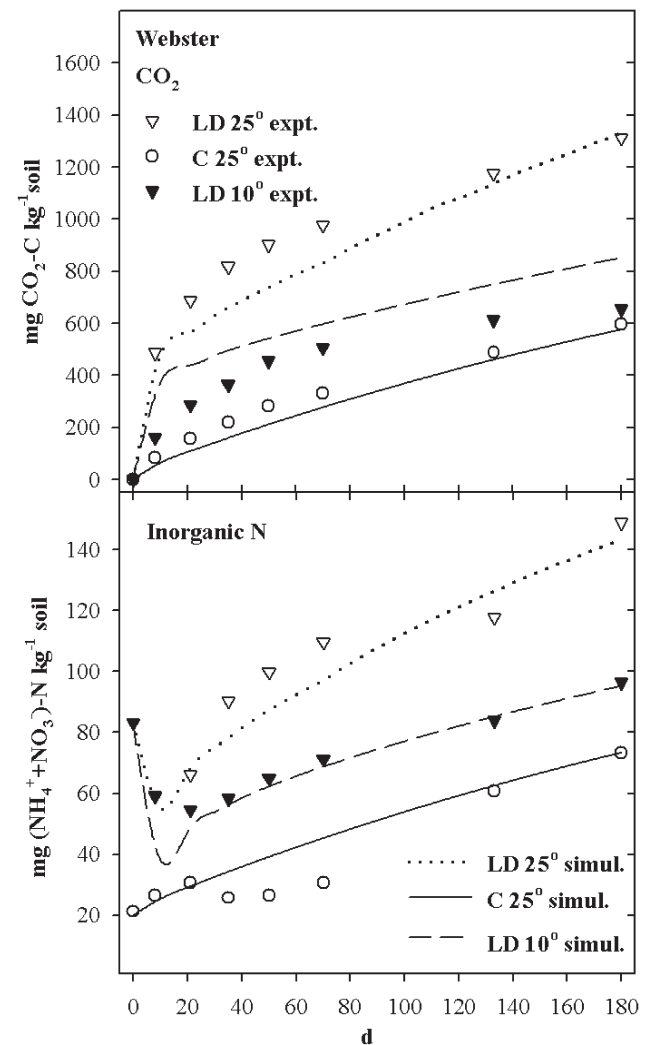


Fig. 5. Experimental (symbols) and simulated (lines) values of cumulative CO₂ and inorganic N of Webster soil amended with liquid dairy manure (LD) and unamended control (C) during incubation at 25 or 10°C.

example were observed, which raises a question: What was the source of N mineralized following the phase of net N immobilization? Remineralization of previously immobilized N by the microbial biomass was one possibility since inorganic N in the form of NO_3^- increased and MBN declined between Days 8 and 180 in the LD 25°C treatments. A mass balance analysis of ^{15}N in the MBN and inorganic N pools, however, showed that the MBN decrease between Days 8 and 35 could not account for the increase in NO_3^- concentration to the level of the control soil during this time. Only a small part of the previously immobilized NH_4^+ was remineralized in this time frame (Fig. 3). After a 3-yr study involving ^{15}N -labeled NH_4^+ in manure, Sørensen and Amato (2002) similarly concluded that the immobilized NH_4^+ -N is slowly released for many years. Therefore, the increase in N_m to the level of the control soil between Days 8 and 35 was probably mainly from mineralization of the N_{org} fraction of the manure.

In the T manure, N_m was similar as in the control soil except at the first two samplings (Fig. 2). Therefore, almost all the NH_4^+ initially present was available after the application, but apparently no N of the N_{org} fraction became available during 180 d. Kirchmann (1991) observed no net mineralization in aerobic poultry manure. Preusch et al. (2002), on the other hand, reported a small net N mineralization of 1 to 5% of the initial organic N in composted poultry litter applied to a silt loam soil. Fresh poultry manure contains a significant amount of uric acid that is rapidly transformed into NH_4^+ (Kirchmann, 1991; Thomsen, 2004). In the T manure of the present study, this conversion of uric acid to NH_4^+ had probably already taken place during storage.

In contrast to the LD, T, and H treatments, N_m in the SD treatment exceeded net mineralization of the control. By Day 180, 33 mg N kg^{-1} more than in the control soil was mineralized, an amount equivalent to 22% of the N_{org} of the SD manure. In comparison, first-year N mineralization of beef cattle feedlot manure during 130 d at a similar temperature was 18% of the N_{org} fraction and 12% of N_{org} of composted

feedlot manure (Eghball, 2000). Kirchmann (1991) observed no net mineralization when fresh or aerobically treated (composted) dairy manure, consisting only of feces, was applied to soil. The SD of the present study contained straw, however, and under the aerobic storage conditions, mineralization and microbial N immobilization may have already started before the manure was collected. Therefore, the SD manure may have had characteristics of a composted product. The net mineralization rate of the SD was also comparable to values of N mineralization after application of composted cattle manure reported in other studies (Castellanos and Pratt, 1981; Chèneby et al., 1994; Hadas and Portnoy, 1994; Paul and Beauchamp, 1994; Shi et al., 1999). In contrast to LD, H, and T, crop-available N from the SD could not have been predicted from the initial NH_4^+ concentration, which was much lower in SD than in the other manure types. Ammonia volatilization, which may have occurred under storage conditions (Misselbrook and Powell, 2005), could have been partially responsible for the low initial NH_4^+ content in this manure.

Net N mineralization was slower at the lower temperature. In the LD 10°C treatment, the N_m concentrations were lower than those of the control for 133 (Tara) or 180 d (Webster) (Fig. 2). The CO_2 emission rates also indicate lower but sustained microbial activity at the lower temperature, which is in agreement with other organic matter decomposition studies (Devêvre and Horwath, 2000; Sims, 1986).

There was less biological activity in Webster than in Tara soils, which may have been due to management history. The Webster soil had not received inputs of any kind since 1986. Soil properties, such as pH, which is influenced by inputs, may have also affected biological activity in the two soils. During the 180-d incubation, MBN in the Tara soil ranged from 58 to 25, and from 34 to 16 mg N kg^{-1} in the Webster soil (Fig. 1). Overall, in both the LD-amended and control soils, MBN was significantly lower in the Webster than in the Tara soil ($P < 0.001$, paired *t*-test). Furthermore, nitrification of the initially present NH_4^+ in the manure-amended Webster soil was slower than in the Tara soil. After 180 d, however, the Webster soil amended with LD displayed greater NO_3^- accumulation above the control than the LD-amended Tara soil at both 10 and 25°C, which was probably due to lower N demand by the microbial biomass in this soil.

Table 2. Cumulative N_2O and NO production (standard errors in parentheses) during the first 63 d of the incubation in Tara and Webster soils amended with liquid dairy manure (LD) at 25 or 10°C, solid dairy manure (SD), turkey manure (T), and liquid hog manure (H) and the unamended control, and the total denitrification rate ($\text{N}_2\text{O}_{\text{AC}}$) on Day 7 of the incubation.

Treatment	N_2O	NO	$\text{N}_2\text{O}_{\text{AC}}$
	— $\mu\text{g N kg}^{-1}$ —		
Tara			
LD, 25°C	64 (11)	540 (21)	1.5 (0.3)
LD, 10°C	141 (73)	754 (261)	
SD	126 (64)	557 (44)	7.0 (0.3)
T	51 (3)	605 (31)	0
Control, 25°C	15 (3)	685 (35)	0.3 (0.1)
Control, 10°C	11 (1)	237 (26)	
Webster			
LD, 25°C	73 (12)	651 (81)	
LD, 10°C	189 (28)	739 (76)	
H	68 (11)	1448 (96)	1.1 (0.3)
Control, 25°C	17 (2)	616 (31)	
Control, 10°C	7 (3)	401 (24)	

Nitrous and Nitric Oxide Production and Denitrification

The N_2O and NO production were measured for the first 63 d, after which they decreased to below detectable levels. During this time, N_2O production was <0.13% of the manure N input for all treatments (Table 2). Nitric oxide was greater than N_2O production. Our flow-through system allowed measurement of gross NO production, whereas in a field situation, some of this NO might have been consumed to yield NO_3^- (Venterea et al., 2004). Cumulative NO production was highest in the soil of the H treatment, where about 1.5 mg NO-N kg^{-1} , roughly twice as much as in the unamended soils, was produced. The amount of NO-N produced in the H treatment was equivalent to about 1% of the total N or 1.5% of the inorganic N in the manure. The N_2O and NO production rates in the present study were comparable to those

measured by Paul et al. (1993) who reported N_2O emissions between 0.025 and 0.85% and NO emissions of about 0.26% of the added manure N during 6 d. Total denitrification, measured by the acetylene inhibition method after 1 wk of incubation, was at most $1.5 \mu\text{g N}_2\text{O-N kg}^{-1} \text{d}^{-1}$. Our measurement of N_2O with and without acetylene inhibition at a time when conditions other than O_2 diffusivity were potentially conducive for denitrification, confirmed that denitrification had little influence on the fate of N in our microcosms. The water-filled pore space in our microcosms was well below 60%, which is considered to be a threshold above which denitrification starts to occur (Linn and Doran, 1984). Preliminary tests before the actual incubation had confirmed this rule for these particular soils and amendments. Another reason for the probable low denitrification rates in the present study may have been the method of manure application. The uniform distribution of homogenized manure slurry probably prevented the formation of “hot spots” of microbial activity where O_2 depletion might occur (Nielsen and Revsbech, 1998). Calderón et al. (2004 and 2005) reported much higher denitrification and N_2O production rates from a soil in which manure was placed in litterbags at a single location. Nonetheless, while denitrification and N_2O production in the current study were probably inhibited by high O_2 availability, the increased NO_3^- availability together with increased available C would increase the potential for denitrification under field conditions in response to higher soil moisture.

Ammonia volatilization, which was not measured, was probably low since the jars were closed most of the time and the manure was in close contact with and covered by soil. Under field conditions, NH_3 losses may be more of a concern.

Simulation Modeling

The LD and SD treatments were chosen for simulation modeling because, in these treatments, potential crop-available N varied due to N immobilization and mineralization of manure N_{org} . For the H and T manures, inorganic N availability corresponded to the initial NH_4^+ content of the manure and background soil N dynamics. In the LD treatments, the simulated inorganic N data agreed closely with the experimental values, e.g., the rapid decline within the first 8 d of the incubation and the lower N mineralization rate at 10°C than at 25°C (Fig. 4 and 5). In the LD 10°C treatment, however, the modeled CO_2 values were lower than the experimental one in the Tara soil, whereas in the Webster soil, the reverse pattern was observed.

The soil temperature used for simulation modeling based on long-term averages at the 10-cm depth in Waseca, MN, during October is 10°C . Between November and March, the average values are close to or below 0°C . Therefore, in this context, incorporation at soil temperatures $>10^\circ\text{C}$ can be considered an “early fall” application, while incorporation just before freezing might be considered a “late fall” application. Simulated by NCSOIL, LD or SD incorporation on 30 September and 31 October resulted in NO_3^- levels of 27 to 33 and

2 to 5 $\text{mg NO}_3^- \text{N kg}^{-1}$, respectively, in March and April (Fig. 6). Thus, early fall incorporation would result in higher NO_3^- pools at the time of snowmelt when leaching takes place. Randall et al. (1999) found an average decrease of about 5% in grain yield with fall compared with spring liquid dairy manure application. This was attributed to possible NO_3^- leaching losses after fall applications because yields and NO_3^- concentrations in the spring were increased if a nitrification inhibitor was used. With late fall applications, the risk of N losses appears to be lower. According to the simulation, $\text{NO}_3^- \text{N}$ concentrations equivalent to the initial $\text{NH}_4^+ \text{N}$ concentration, which could be used as a first-year predicted value of crop-available N from LD, were reached by 1, 18, and 25 June and 3 July after early fall (30 September), late fall (31 October), early spring (15 April), and late spring (5 May) incorporation, respectively. Nitrate availability would not be much later with spring than with late fall application, which is often preferred by growers from an operational perspective (Randall et al., 1999). Crop N demand for corn is at a maximum in June (McWilliams et al., 1997). Therefore, spring manure application should be as early as possible for maximum benefit to a crop. With LD, as much $\text{NO}_3^- \text{N}$ as measured in the NH_4^+ fraction could be expected to be available within 6 wk. According to the SD simulation, similar $\text{NO}_3^- \text{N}$ concentrations as in the LD treatments were reached about 3 wk later than with LD application for each respective manure incorporation date.

The incubation data and the results of the simulation showed that N mineralization was temperature dependent to a greater degree than N immobilization. Therefore, it could be expected that a substantial part of NH_4^+ in manure would be rapidly immobilized even with late fall incorporation of a

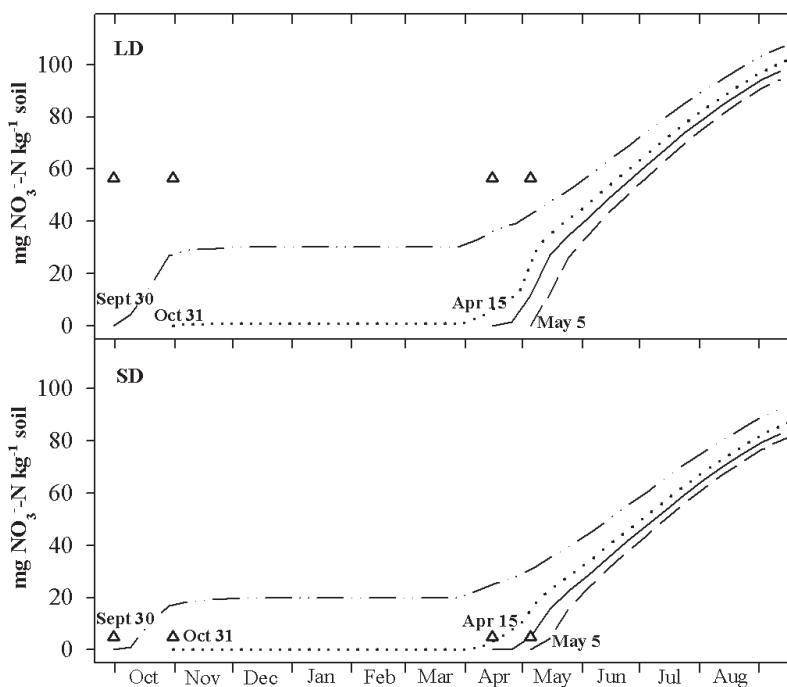


Fig. 6. Ammonium concentration (symbols) of the liquid dairy manure (LD) and solid dairy manure (SD) (total N input 154 mg N kg^{-1}) and simulated soil NO_3^- concentrations (lines) after incorporation in Tara soil on different dates, using long-term soil temperature averages from southern Minnesota and assuming a soil moisture content of 60% water holding capacity.

manure type with high immobilization potential, such as LD. On the other hand, under field conditions, soil water movement shortly after application of the manure slurry, e.g., by rainfall, might separate organic matter from NH_4^+ , and thus prevent NH_4^+ immobilization. Previous studies of temperature effects on N mineralization-immobilization kinetics support this view. Nicolardot et al. (1994a) also reported N immobilization of 20 mg kg^{-1} within <10 d even at 4°C if a readily available C source was present, but net N immobilization was about 50% greater and more rapid at 28°C. Cookson et al. (2002) observed increases in gross N immobilization with temperature changes from 15 to 2°C and from 2 to 15°C after 98 d incubation of clover (*Trifolium repens* L.)-amended silt loam soil at 15 or 2°C, but gross N mineralization increased only when the temperature was increased from 2 to 15°C. Thus, N mineralization was more sensitive than N immobilization to temperature change. According to our simulation, late fall incorporation lowers mineralization before spring thaw. The effectiveness of late fall application to minimize mineralization may vary from year to year due to the occasional occurrence of warming periods during the winter months. While NCSOIL assumes no microbial activity at <0°C, studies have shown measurable microbial activity at much lower temperatures (Panikov et al., 2006). Much of the below 0°C research on microbial activity has been performed in arctic and alpine soils, and little is known about the relative importance of mineralization at <0°C in temperate regions.

The calibrated model NCSOIL simulated C and N dynamics reasonably well; however, the proportion of the C pools and the respective C/N ratios of the manure organic fractions were chosen based on model optimization. Therefore, predictions of inorganic N availability using NCSOIL based solely on the manure parameters measured in this study would probably not be reliable. For example, the N immobilization potential of each manure type was not predictable based on the manure properties measured. Besides volatile fatty acids, cell wall fractions might enhance N immobilization (Van Kessel et al., 2000).

CONCLUSIONS

In LD, H, and T treatments, there was good agreement between initial NH_4^+ content and inorganic N available from manure within 180 d. Therefore, for these manure types, estimates of first-season available N might be improved by measuring manure NH_4^+ in addition to total N, which must be determined to keep track of total N inputs into farmers' fields. The SD manure, which had a low initial NH_4^+ content, was the only manure type that showed net N mineralization of the organic N fraction. Immobilization of NH_4^+ was most apparent in the two slurries, LD and H. In the LD treatment, NH_4^+ immobilization was rapid at both 25 and 10°C, whereas mineralization was slower at the lower temperature. Thus, microbial N immobilization was less temperature sensitive than mineralization and nitrification. Based on the incubation and modeling results, under U.S. Upper Midwest climate conditions, NO_3^- pools at the time of snowmelt would be smaller with late fall than with early fall incorporation because immobilization of NH_4^+ in manure, such as LD, and low soil temperatures contribute to delayed nitrification. The simulation modeling

with NCSOIL also showed that C pools of manure should be better characterized with regard to their quantity and availability. Such information could be used in developing indices that would enable models, such as NCSOIL, to predict net N immobilization and mineralization kinetics based on manure properties measured before application to the soil.

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