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DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Carbon and Nitrogen Dynamics During Incubation of Manured Soil

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ABSTRACT

Denitrification N losses during manure net mineralizable N assays may lead to miscalculation of the manure's N-supplying capacity. In this study we measured denitrification, manure properties, gas fluxes, nutrient pools, and mineralizable N during laboratory incubation of manured soil. Different dairy manures (n = 107) were added to soil at a rate of 0.1 mg N g^{-1}. Manured and control soils were incubated and sampled weekly for soil mineral N, CO_2 flux, and N_2O flux. The denitrification enzyme activity (DEA) was measured at the end of the experiment. Weekly N_2O and CO_2 production increased in the manured soils during the first 3 wk of incubation. There was a positive correlation between added manure C and cumulative CO_2 production. Nitrate content increased in all soils throughout the 6-wk period, but the increase was more marked in the manured soils. In most manured soils, ammonium concentration was initially high then declined rapidly during the first 2 wk. This high net NH_4^+ decline in the manured soils suggests that N was immobilized during the incubation. Microbial biomass N should be determined during manure mineralization assays to account for all potential manure N sinks. No correlation existed between DEA and N pools or gas fluxes in the manured soils. Manures with negative N mineralization had an average C/N of 19.0, while manures with positive N mineralization had an average C/N of 16.0. On average, denitrification accounted for approximately 5% of the added manure N. Higher proportions of denitrified N were observed in some manures, supporting our hypothesis that N losses through denitrification may be significant in manure mineralizable N assays.

THE QUALITY OF A MANURE as an N fertilizer is difficult to predict, since manure composition may vary widely according to diet and other management factors (Reeves and Van Kessel, 2002; Van Kessel and Reeves, 2002). Dairy manure is a complex mixture of materials with varied mineralization kinetics ranging from relatively resistant lignin to readily available ammonium and volatile fatty acids (VFA) (Van Kessel et al., 2000).

Laboratory incubations are performed to determine the N mineralization potential of manure-amended soils (Bernal and Kirchmann, 1992). Incubation studies have shown that the mineralization potential of manures may be related to manure C/N ratio and manure N content (Jedidi et al., 1995). According to incubation studies, some manures act as net suppliers of N, while others may result in net N immobilization. Hadas and Portnoy (1994) determined that manures might release up to 29% of their N content as inorganic N during a laboratory incubation. In contrast, Sørensen (1998) found net immobilization of N during manure incubations, and the effect was pronounced with manures rich in VFA content. In addition, N losses through denitrification as well as N immobilization during laboratory incubations may affect the correlation of incubation data with manure N mineralization in the field, as well as preventing a good agreement between manure N pools and mineralizable N.

We hypothesize that denitrification may divert manure mineralizable N to N gas, resulting in inaccurate estimation of mineralizable N during laboratory incubations. Because of this, accounting for denitrified N may improve the correlation between manure N and mineralizable N. Farmers and extension agents need accurate information regarding manure N availability to make sound manure management decisions. Previous work to determine denitrification N losses after manure application has included few manures per study. However, to better represent field variability, it is necessary to examine and compare a large number of manures. In this study, we collected manures from a wide variety of farms each with different storage conditions, resulting in a set of manures that varied widely in composition.

The objectives of this experiment were to: (i) determine the effect of manure addition on soil C and N dynamics; (ii) determine if manure chemical or nutritional properties correlate to C and N pools, gas fluxes or denitrification during a laboratory incubation of manured soil; and (iii) measure the amount of N lost through denitrification during a mineralizable N assay. To achieve these objectives, we performed 6-wk laboratory incubations of manured soils. Soil mineral N, CO_2 flux, and N_2O flux after acetylene addition were measured weekly. Manure properties were measured before the incubation experiment, while DEA was measured at the end of the 6-wk incubation.

MATERIALS AND METHODS

Soil and Manure Collection

The Christiana fine sandy loam soil (typic Normudults) used in this study was obtained from an alfalfa field located on the USDA-ARS Beltsville Agricultural Research Center. The soil was obtained from the Ap horizon and had an organic C of 19.3 mg g^{-1}, and total N content of 1.6 mg g^{-1}. Clay, silt,

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Published in Soil Sci. Soc. Am. J. 68:1592–1599 (2004).

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Abbreviations: ADF, acid detergent fiber; DEA, denitrification enzyme activity; FDM, dry matter fiber; NDF, neutral detergent fiber; VFA, volatile fatty acids.

Table 5. N_2O-N production and CO_2–C production during the denitrification enzyme activity (DEA) assay measured at the end of the 6-wk incubation. n = 107 for the manured soil. n = 6 for the control soils.

	Average (SEM)	
Variable	Manured soils	Control soils
	mg kg^{-1} h^{-1}	
N_2O	0.34 ^{a} (0.01)†	0.24 ^{b} (0.02)
CO_2	2.08 ^{a} (0.09)	1.82 ^{a} (0.23)

† Means not sharing a letter within a row are statistically different according to ANOVA ($p < 0.01$).

characterized by very small N_2O fluxes as well as low DEA relative to the manure treatments. The positive correlation between initial soil nitrate and DEA suggests that denitrification was limited by nitrate availability in the control soils. The fact that acetylene did not stimulate N_2O production further supports that denitrification was negligible in the controls. However, the correlation between cumulative CO_2 and cumulative N_2On in the controls suggests a relationship between microbial activity and N oxide fluxes.

The observed N_2Oa, as well as the increased DEA in the manured soils indicates that denitrifiers were favored by manure addition. We hypothesize that the increased nitrate and assimilable C in the manured soil combined to produce anaerobic microsites with increased denitrification activity. The lack of correlation between N_2O fluxes and NO_3^- may be due to a rapid C and N cycling that did not allow pool sizes to represent nutrient availability. The combined low N_2O fluxes and high NO_3^- concentration during the last 3 wk of the incubation suggests that denitrifiers were C-limited during this time period.

Denitrification rates usually reach maximum values between water filled pore spaces of 60 to 100% (Groffman and Tiedje, 1988). In this study, average water filled pore space ranged from 23.1 to 25.5%. Because of this, we hypothesize that the denitrification rates reported in this study are moderate, and higher moisture contents may have resulted in proportionately higher denitrification N losses.

Manure Variables and Nitrogen mineralization

It is possible that measurement of more specific manure organic N fractions may have improved our understanding of the relationship between manure N content and manure mineralizable N. Our analysis of manure N pools was limited to total manure N, manure NH_4–N, and manure organic N. Other forms of manure N such as proteins or amino acids were not analyzed. Alternatively, the lack of correlation between manure N pools and manure N mineralization could have improved by including a measure of immobilized N. Because of this, future studies should include measurements of microbial biomass N throughout the incubation, as well as more specific manure N pools.

Including the lignin/N ratio as an independent variable may improve the estimate of the amount of mineralizable N (Vigil and Kissel, 1991). The ADF, NDF, and lignin in dairy manure are a result of undigested forage cell walls, with each fraction having different mineralization properties (Van Kessel et al., 2000; Van Kessel and Reeves, 2002). Our data shows that none of the fiber variables (ADF, NDF, or lignin) showed a strong correlation with C mineralization or N mineralization during the incubation, suggesting that these fractions contain relatively resistant C and do not significantly affect net nutrient immobilization or mineralization. Alternatively, it is possible that these manure components are only part of a multivariate set of parameters that take part in the mineralization kinetics and simple correlations will not illustrate their importance.

Beauchamp and Paul (1989) suggested that manures with C/N ratios below 15 are likely to result in positive N mineralization after application to soil. In this study, the C/N ratio does play a role in the mineralization characteristics of the manures, since manures associated with positive N mineralization had a mean C/N of 16.0, while manures associated with negative N mineralization had on average a C/N of 19.0.

Volatile fatty acids are part of the water-soluble manure C that is readily accessible to microbial utilization (Paul and Beauchamp, 1989b; Kirchmann and Lundvall, 1993). It has been shown that VFA serve as C sources for denitrifiers, leading to increased nitrogen oxide and CO_2 fluxes from manure-amended soils (Paul and Beauchamp, 1989a, 1989b). The VFA are important C sources and favor O_2 consumption by soil microbes, creating beneficial conditions for denitrifiers in recently manured soils (Paul and Beauchamp, 1989a, 1989b). However, in this study, the lack of correlation between VFA and N pools suggest that other sources besides VFA play a role as C sources for denitrifiers. The lack of correlation between manure VFA and net N mineralization contrast with the findings of Kirchmann and Lundvall (1993), who found a strong correlation of VFA and negative N mineralization after application of animal

Table 6. Summary of stepwise selection of the stepwise regression procedure. The analysis was performed using the STEPWISE Model-Selection of the PROC REG procedure of SAS version 8.2 (Cary, NC).

Step	Variable	Units	Partial R^2	Model R^2	C_p	F value	Pr. > F
1	isobutyric acid	mg g^{-1}	0.10	0.10	85.05	24.00	<0.0001
2	manure H_2O content	%	0.05	0.15	72.09	11.23	0.001
3	manure NH_4–N	mg g^{-1}	0.08	0.23	47.78	21.72	<0.0001
4	manure N/N ratio		0.08	0.31	24.02	23.58	<0.0001
5	propionic acid	mg g^{-1}	0.03	0.34	15.34	10.22	0.002
6	lignin	%	0.02	0.36	11.64	5.57	0.019
7	isovaleric acid	mg g^{-1}	0.02	0.37	8.76	4.85	0.029
8	acid detergent fiber	%	0.01	0.39	6.19	4.63	0.033
9	manure organic N	%	0.01	0.40	4.75	3.53	0.062
10	manure total C	%	0.01	0.41	4.04	2.82	0.095

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headspace autosampler (Tekmar Co., Cincinnati, OH) and a Tremetrics Model 540 gas chromatograph (Tremetrics Inc., Austin, TX), using the conditions detailed in McCarty and Blicher-Mathiesen (1996). Gas samples for N_2O analysis were obtained in the same fashion and at the same time as the CO_2 samples. The N_2O analysis was performed with a Tekmar 7000 HT headspace autosampler (Tekmar Co., Cincinnati, OH) in series with a Shimadzu GC-8A (Shimadzu Scientific Instruments, Inc., Columbia, MD) fitted with an ECD detector.

Soil Mineral Nitrogen

Soil samples (10 g fresh weight) from each microcosm were analyzed for extractable soil mineral N using the same procedure and instruments as for the manure samples (see above). A ratio of 10 g of soil to 50 mL of 2*M* KCL solution was used for the extraction. In this study, the term net N mineralization is used as the change in the soil inorganic N over the 6-wk incubation (Hart et al., 1994). Previous work has referred to negative N mineralization as N immobilization, even when microbial biomass N was not measured directly (Kirchmann and Lundvall, 1993). We will instead refer to negative net N mineralization as apparent N immobilization, since denitrification N losses often do not account for the decline in soil mineral N during the incubation.

Denitrification Enzyme Activity

Duplicate soil samples (four per manure) were analyzed from the Week 6 microcosms using a modified procedure for DEA (Tiedje, 1994). Soil samples (20 g fresh weight) were placed in 100 mL serum bottles and 20 mL of a slurry solution (0.4 g KNO_3, 5.0 g glucose, 1.0 g chloramphenicol, in 1 L H_2O) were added to each serum bottle. The bottles with the soils and slurry mixtures were then capped with butyl rubber septa and crimp caps. Anaerobic conditions were created by alternatively applying vacuum pressure and purging with He gas. Three milliliters of C_2H_2 were then added to each bottle followed by vigorous shaking (1 min.). The serum bottles were then placed in a shaker (200 rpm). The headspace gas was sampled at 1 and 3 h. The N_2O gas samples (1 mL) and the CO_2 gas samples (2 mL) were then stored and analyzed using the same procedure and instruments as the CO_2 and N_2O fluxes (see above).

Statistical Analysis

The PROC CORR of SAS version 8.2 (Cary, NC) was used to determine the Pearson correlation coefficients (r) among the manure properties and the incubation data, as well as the probability (p) that the correlation coefficient was different from zero. When determining the Pearson correlation coefficients, the data of the controls was analyzed separately from the data of the manured microcosms. The PROC GLM procedure of SAS was used to carry out Analysis of Variance (ANOVA) to test effects of manure addition (manured vs. control), time (week), and set. All the variables regarding the mineral N pools as well as gas fluxes during the incubation were included as dependent variables. In addition, mean separations were performed using the least significant difference (LSD) test based on a t test.

We performed multiple regressions using the PROC REG procedure of SAS version 8.2 (Cary, NC). Manure variables such as dry matter, C, total N, Organic N, P_2O_5, K_2O, NH_4–N, lignin, ADF, NDF, as well as the individual VFA were included as dependent variables. The Forward Selection (FORWARD), Stepwise (STEPWISE), Maximum R^2 improvement (MAXR), and Full Model Fitted (NONE) Model-Selection methods of the PROC REG procedure were used. By performing the different Model-Selection methods, we aimed to establish if a combination of manure properties explains a proportion of the variance of the manure mineralizable N, and also establish the relative predictive importance of the independent variables.

RESULTS

Manure Properties

Manure C/N ratios ranged widely from 11.29 to 38.88 (Table 1). As expected, C rich components such as NDF (r = −0.73) and ADF (r = −0.65) were negatively correlated with manure percentage of N (Table 2). Neutral detergent fiber was negatively correlated with manure ammonium content (r = −0.60; Table 2).

Seven different VFA were detected and quantified in the manure samples (Table 3). Individual VFA were correlated among themselves, with R scores ranging from 0.53 to 0.98 (data not shown). However, none of the manure VFA correlated strongly with any of the other manure properties or incubation variables.

Carbon Dioxide Fluxes

The addition of manure increased the CO_2 flux of the soils (Fig. 1). The largest difference between manured and control soils occurred at Week 1, when the manured soils had from 42 to more than 400% higher CO_2 fluxes. Cumulative C mineralization (as CO_2 flux) during the 6-wk incubations averaged 1.38 g kg^{-1} in the manured

Table 2. Correlation matrix for selected variables for all the manured soils. The control treatment was excluded from the analysis.†

	Man. % N	Man. NH_4–N	Added Man. C	ADF	NDF	Wk 0 NH4	Wk 6 NO_3	Cum. N_2Oa	Cum. N_2On	Cum. CO_2	Net N miner.	Immob.
Man. % N	1.00	0.43	−0.50	−0.65	−0.73	−0.04	0.18	−0.15	−0.07	−0.42	0.20	0.16
Man. NH_4–N	–	1.00	−0.60	−0.56	−0.60	0.51	0.43	−0.30	−0.20	−0.46	−0.27	−0.35
Added Man. C	–	–	1.00	0.46	0.62	−0.21	−0.28	0.32	0.13	0.69	0.02	0.07
ADF	–	–	–	1.00	0.87	−0.25	−0.20	0.09	0.03	0.36	0.11	0.12
NDF	–	–	–	–	1.00	−0.15	−0.21	0.21	0.10	0.52	0.00	0.04
Wk 0 NH_4	–	–	–	–	–	1.00	0.41	−0.04	0.12	0.00	−0.81	−0.72
Wk 6 NO_3	–	–	–	–	–	–	1.00	−0.14	−0.20	−0.17	−0.20	−0.29
Cum. N_2Oa	–	–	–	–	–	–	–	1.00	0.37	0.20	0.00	0.17
Cum. N_2On	–	–	–	–	–	–	–	–	1.00	0.13	−0.13	0.33
Cum. CO_2	–	–	–	–	–	–	–	–	–	1.00	−0.23	−0.16
Net N miner.	–	–	–	–	–	–	–	–	–	–	1.00	0.89
Immob.	–	–	–	–	–	–	–	–	–	–	–	1.00

† Abbreviations are as follows: Manure is man., cumulative is cum., and mineralization is miner. Apparent immobilization (Immob) is the net N mineralization minus the cumulative N_2On. Correlation coefficients above an absolute value of 0.26 have a slope significantly different from zero ($p < 0.001$). n = 107.