Soil Biology & Biochemistry 41 (2009) 348–356

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Nitrogen transformations in cold and frozen agricultural soils following organic amendments

Karen Clark [a], Martin H. Chantigny [a,*], Denis A. Angers [a], Philippe Rochette [a], Léon-Étienne Parent [b]

[a] Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, 2560 Hochelaga Boulevard, Québec, QC, Canada G1V 2J3
[b] Département des sols et de génie agroalimentaire, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec, QC, Canada G1K 7P4

ARTICLE INFO

Article history:
Received 3 September 2008
Received in revised form
4 November 2008
Accepted 17 November 2008
Available online 6 December 2008

Keywords:
Mineralization
Nitrification
Immobilization
Cold temperature
Frozen soils

ABSTRACT

Though microbial activity is known to occur in frozen soils, little is known about the fate of animal manure N applied in the fall to agricultural soils located in areas with prolonged winter periods. Our objective was to examine transformations of soil and pig slurry N at low temperatures. Loamy and clay soils were either unamended (Control), amended with $^{15}NH_4$-labeled pig slurry, or amended with the pig slurry and wheat straw. Soils were incubated at −6, −2, 2, 6, and 10 °C. The amounts of NH_4, NO_3 and microbial biomass N (MBN), and the presence of ^{15}N in these pools were monitored. Total mineral N, NO_3 and $^{15}NO_3$ increased at temperature down to −2 °C in the loam soil and −6 °C in the clay soil, indicating that nitrification and mineralization proceeded in frozen soils. Nitrification and mineralization rates were 1.8–4.9 times higher in the clay than in the loamy soil, especially below freezing point (3.2–4.9), possibly because more unfrozen water remained in the clay than in the loamy soil. Slurry addition increased nitrification rates by 3–14 times at all temperatures, indicating that this process was N-limited even in frozen soils. Straw incorporation caused significant net N immobilization only at temperatures ≥2 °C in both soils; the rates were 1.4–3.4 higher in the loam than in the clay soil. Nevertheless, up to 30% of the applied ^{15}N was present in MBN at all temperatures. These findings indicate that microbial N immobilization occurred in frozen soils, but was not strong enough to induce net immobilization below the freezing point, even in the presence of straw. The Q_{10} values for estimated mineralization and nitrification rates were one to two orders-of-magnitude larger below 2 °C than above this temperature (13–208 versus 1.5–6.9, respectively), indicating that these processes are highly sensitive to a small increase in soil temperature around the freezing point of water. This study confirms that net mineralization and nitrification can occur at potentially significant rates in frozen agricultural soils, especially in the presence of organic amendments. In contrast, net N immobilization could be detected essentially above the freezing point. Our results imply that fall-applied N could be at risk of overwinter losses, particularly in fine-textured soils.

1. Introduction

It is now recognized that microbial activity occurs at sub-zero temperatures in soils at high latitudes (Schimel et al., 2004; Gregorich et al., 2006; Panikov et al., 2006) and high altitudes (Sommerfeld et al., 1993; Brooks et al., 1997). Many agricultural soils are located in areas that experience prolonged winters with sub-zero temperatures. Though denitrification (Bremner and Zantua, 1975; Dorland and Beauchamp, 1991; Chantigny et al., 2002) and nitrification (Malhi and Nyborg, 1979; Malhi and McGill, 1982; Cookson et al., 2002) have been reported in agricultural soils at sub-zero temperatures, little is known about the significance of these microbially mediated N transformations in the context of annual N cycling. Low but sustained microbial activity was reported in soils under snowpack (Sommerfeld et al., 1993; Brooks et al., 1997). Sustained nitrification and N mineralization–immobilization turnover during the winter period could have a significant impact on the fate of N from organic amendments applied in the fall.

Overwinter soil N losses have been reported after application of animal manure or mineral fertilizer in the fall (Bole and Gould, 1986; Nyborg and Malhi, 1986; Nyborg et al., 1990). These losses come partly in the form of NO_3 leaching to groundwater (Cookson et al., 2002; Gupta et al., 2004) and N_2O emission during winter and spring thaw (Christensen and Tiedje, 1990). The incorporation of straw or crop residues with high C-to-N ratios is expected to induce N immobilization (Mary et al., 1996) and mitigate the loss of N from soil. However, N immobilization has been found to be more sensitive to low temperature than nitrification (Hoyle et al., 2006)

* Corresponding author. Tel.: +1 418 210 5013; fax: +1 418 648 2402.
E-mail address: chantignym@agr.gc.ca (M.H. Chantigny).

0038-0717/$ – see front matter Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2008.11.009

or N mineralization (Andersen and Jensen, 2001), though the opposite was observed in the presence of a readily available C source (Cookson et al., 2002). Little is known about the minimum temperature at which various N transformation processes become negligible in agricultural soils in high latitudes.

Pig slurry typically contains 50–60% of its total N as NH_4, which can be rapidly nitrified (Morvan et al., 1997; Rochette et al., 2000; Chantigny et al., 2001) and is subsequently prone to environmental loss. Pig slurry may be applied in the fall, but little is known about the fate of its N at low temperature. Furthermore, the ability of high C-to-N crop residues to immobilize pig slurry NH_4 at low temperatures has not been assessed.

Microbial activity may continue at sub-zero temperatures in the film of water surrounding soil particles (Bremner and Zantua, 1975). Moreover, the amount of unfrozen soil water has been shown to increase with clay content (Stähli and Stadler, 1997), such that microbial activity at sub-zero temperatures could be greater in fine- than coarse-textured soils.

Our objectives were to determine under controlled conditions whether: (i) mineralization, nitrification, and immobilization can occur in frozen agricultural soils from eastern Canada, (ii) pig slurry N can be nitrified and immobilized at low temperatures, and (iii) soil texture influences mineralization, nitrification, and N immobilization at low temperatures.

2. Materials and methods

2.1. Soils and experimental set-up

A heavy Kamouraska clay and a loamy St-André soil were collected from the Harlaka Research Farm of Agriculture and Agri-Food Canada, 5 km south of Québec City (46°48′ N, 71°23′ W), Canada (Table 1). The mean annual air temperature of this area is 4.2 °C and mean annual precipitation is 1213 mm. The soil samples were collected from the plough layer (0–20 cm) in early November 2005, when the average daily air temperature was about 4 °C. The soil samples were sieved to 6 mm and stored field-moist at 4 °C for 3 weeks to allow soil respiration to stabilize. Soil texture was measured with the hydrometer method (Sheldrick and Wang, 1993). Total N and C were measured by dry combustion (Model TruSpec CN, Leco Corp., St-Joseph, MI).

Wheat straw was chopped using a grinder fitted with a 2-mm screen. One hour before addition to soils, 1 L of pig slurry was enriched with ^{15}N by the addition of $(^{15}NH_4)_2SO_4$ (99 at. % ^{15}N) and gently shaken for 15 min to ensure homogeneous distribution of $^{15}NH_4$.

Field-moist samples were weighed (100 g dry basis) and placed in 500-mL glass jars. The samples were either unamended (Control), or received 1.1 mL of pig slurry (delivered with a pipette) alone or in combination with 0.5 g of chopped wheat straw. These amendment rates corresponded to field application of *ca.* 55 kg NH_4-N ha^{-1} as slurry and 10 Mg dry matter ha^{-1} as straw to emulate typical practices in the fall in eastern Canada. After amendment, the soil was thoroughly mixed and distilled water was added, dropwise with a syringe, to bring soil water tension to −6 kPa in all jars. This water tension is representative of fall and winter field conditions in eastern Canada (Chantigny et al., 2002). The glass jars were incubated with aluminium screw-top lids pierced with a 7-mm diameter hole to avoid hypoxia while limiting water evaporation.

The experimental set-up (2 soils × 3 treatments × 3 replicates) was repeated 8 times (total of 144 jars) to allow for destructive sampling after 7, 14, 28, 49, 70, 91, 112, and 133 d of incubation. Five identical sets of 144 jars were prepared and incubated in 5 growth chambers set at either −6, −2, 2, 6, or 10 °C to encompass a range of soil temperatures typically encountered from fall to spring in eastern Canada. Only one growth chamber was used per incubation temperature. Temperature in all chambers was continuously monitored using a type-T copper-constantan thermocouple attached to a datalogger (CR-10X, Campbell Scientific, Logan, UT) to validate accuracy. A double random distribution was used to allot jars to a given chamber (temperature) and to determine the position of each jar in the chamber.

Table 1
Selected characteristics of the soils and amendments.

	pH	Sand ($g\,kg^{-1}$)	Clay ($g\,kg^{-1}$)	Total C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	NH_4-N ($g\,kg^{-1}$)	^{15}N abundance (atom %)
Soils							
Clay	6.27	163	505	38.6	3.17	ND	0.369
Loam	5.95	468	215	21.0	1.48	ND	0.368
Amendments							
Pig slurry[a]	8.26	ND	ND	8.97	2.78	2.18	7.29
Straw	ND	ND	ND	437	7.39	ND	ND

ND, not determined.
[a] This slurry had a density of 1.05 kg L^{-1}.

2.2. Analyses

Selected characteristics of the straw and pig slurry are presented in Table 1. Total C of wheat straw was measured by dry combustion (Model TruSpec CN, Leco Corp., St-Joseph, MI), whereas slurry total C was measured by direct injection (200 μL) of a homogenized sample into an automated dissolved C analyzer (Model Formacs, Skalar Analytical, De Breda, The Netherlands). Total N in both amendments was measured by acid digestion using H_2SO_4–H_2SeO_3 (Isaac and Johnson, 1976) and determination of NH_4 concentration with an automated continuous flow colorimeter (Model QuickChem 8000 FIA+, Lachat Instruments, Loveland, CO). The ^{15}N abundance in pig slurry was determined on an aliquot of the acid digest (enough to provide *ca.* 100 μg N) neutralised to pH 6 with 2 M NaOH, and evaporated to dryness at 50 °C. The precipitate was transferred into a tin capsule to determine ^{15}N abundance by mass spectrometry as detailed below for soils. Pig slurry NH_4 content was determined after shaking 5 mL of the slurry with 120 mL of 2 M KCl for 30 min, centrifuging (15,000 × *g*; 10 min) and filtering through filter paper (Whatman #42) pre-washed with 2 M KCl. The NH_4 present in the KCl extract was quantified as described above for the acid digests.

Soil mineral N was determined by extracting 30 g of fresh soil with 120 mL of 2 M KCl. Soil slurries were shaken in 250-mL polypropylene bottles for 60 min (reciprocal shaker; 120 strokes per min), centrifuged for 10 min (3000 × *g*) and filtered on papers (Whatman #42) pre-washed with 2 M KCl. Soils in jars incubated at −2 and −6 °C were a solid mass at the time of each sampling and had to be thawed at room temperature for about 30 min before subsampling and extraction. Concentrations of NH_4 and NO_3 in the extracts were determined with the automated colorimeter mentioned above for total N in amendments.

Soil microbial biomass N (MBN) was determined with the fumigation–extraction method (Voroney et al., 1993) after 14, 70 and 133 d of incubation. Two 20-g samples were collected from each jar; one was fumigated with ethanol-free $CHCl_3$ in a desiccator under vacuum for 24 h, whereas the other was immediately extracted. Both sub-samples were transferred into 250-mL centrifugation bottles, extracted with 40 mL of 0.25 M K_2SO_4 for 30 min on a reciprocal shaker, centrifuged for 10 min (3000 × *g*), and filtered on glassfibre filter paper (Whatman #934-AH). Total N concentration in K_2SO_4 extracts was measured by oxidation with potassium persulfate and NO_3 concentration was determined following oxidation (Cabrera and Beare, 1993). Soil MBN was