Soil Biology & Biochemistry 40 (2008) 2553–2562

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

# Crop residue influence on denitrification, $N_2O$ emissions and denitrifier community abundance in soil

M.N. Miller [a,b], B.J. Zebarth [a,*], C.E. Dandie [a,1], D.L. Burton [c], C. Goyer [a], J.T. Trevors [b]

[a] Potato Research Centre, Agriculture and Agri-Food Canada, P.O. Box 20280, Fredericton, NB, Canada E3B 4Z7
[b] Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1
[c] Department of Environmental Sciences, Nova Scotia Agricultural College, Truro, NS, Canada B2N 5E3

ARTICLE INFO

*Article history:*
Received 11 February 2008
Received in revised form 19 June 2008
Accepted 21 June 2008
Available online 25 July 2008

*Keywords:*
Carbon
*cnorB* gene
Denitrifying enzyme activity
$N_2O$ molar ratio
Nitrous oxide
*nosZ* gene
Real-time PCR

ABSTRACT

Bacterial denitrification plays an important role in the global nitrogen cycle and is a principal contributor of nitrous oxide ($N_2O$) to the atmosphere. The influence of simple (glucose) and complex (red clover and barley residue) carbon (C) sources on the amount of denitrification, $N_2O$ molar ratio ($N_2O$:($N_2 + N_2O$)), and abundance of soil total bacterial and denitrifier communities was investigated using repacked soil cores. Quantitative PCR was used to determine the abundance of the total bacterial community (16S rRNA gene) and components of the denitrifier community, $cnorB_P$ (*Pseudomonas mandelii* and related species), $cnorB_B$ (*Bosea/Bradyrhizobium/Ensifer* spp.) and *nosZ* gene bearing communities. The relationship between the supply of, and demand for, terminal electron acceptors (TEAs), as determined by the relative availability of C and nitrate ($NO_3^-$), influenced the amount of denitrification and the $N_2O$ molar ratio for both simple and complex C sources. Addition of glucose and red clover to the soil increased microbial activity, leading to $NO_3^-$ depletion and an increased consumption of $N_2O$, whereas in soil amended with barley straw, there was not sufficient stimulation of microbial activity to create sufficient TEA demand to cause a measurable increase in emissions. This resulted in a higher $N_2O$ molar ratio at the end of the incubation for the barley straw amended soil. A significant relationship ($R^2 = 0.83$) was found between respiration and cumulative denitrification, suggesting that the available C increased microbial activity and $O_2$ consumption, which led to conditions favorable for denitrification. The source of C did not significantly affect the total bacterial community or the *nosZ* copy numbers with an average of $4.9 \times 10^7$ 16S rRNA gene copies $g^{-1}$ dry soil and $4.6 \times 10^6$ *nosZ* gene copies $g^{-1}$ dry soil, respectively. The addition of red clover plus $NO_3^-$ significantly increased the $cnorB_P$ denitrifier community in comparison with the unamended control while the density of the $cnorB_P$ denitrifier community increased from $3.9 \times 10^4$ copies $g^{-1}$ dry soil to a maximum of $8.7 \times 10^5$ copies $g^{-1}$ dry soil following addition of glucose plus $NO_3^-$ to soil. No significant correlations were found between the denitrifier community densities and cumulative denitrification or $N_2O$ emissions, suggesting that the denitrification activity was decoupled from the denitrifier community abundance.



## 1. Introduction

Biological denitrification is central to the global nitrogen cycle. It is a dissimilatory process whereby nitrate ($NO_3^-$) and nitrite ($NO_2^-$), are used as alternative electron acceptors and reduced to gaseous nitric oxide (NO), nitrous oxide ($N_2O$) and molecular nitrogen ($N_2$). $N_2O$ is an intermediate in the denitrification process, and consequently both the amount of denitrification and the $N_2O$ molar ratio ($N_2O$:($N_2 + N_2O$)) are important in understanding and predicting $N_2O$ emissions. The $N_2O$ molar ratio is variable in space and time and this is of concern because $N_2O$ is a greenhouse gas and a catalyst of stratospheric ozone degradation (Crutzen, 1981).

Both the rate of denitrification and the $N_2O$ molar ratio in soil are regulated by various environmental factors including soil water content, temperature, soil pH, redox potential, nitrogen oxide concentrations and availability of carbon (C) (Firestone and Davidson, 1989; Hutchinson and Davidson, 1993). Denitrification is generally promoted under high soil moisture conditions where oxygen is limited, and $NO_3^-$ and organic C are available for denitrifying microorganisms (Luo et al., 1999).

Carbon availability is one of the most important factors controlling denitrification rates (Beauchamp et al., 1989). It is of interest because it generally increases the amount of denitrification

* Corresponding author. Tel.: +1 506 452 4828; fax: +1 506 452 3316.
*E-mail address:* zebarthb@agr.gc.ca (B.J. Zebarth).
[1] Present address: Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, SA 5095, Australia.

0038-0717/$ – see front matter Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2008.06.024

while either decreasing (Weier et al., 1993; Mathieu et al., 2006) or increasing (Dendooven et al., 1996; Mathieu et al., 2006) the $N_2O$ molar ratio. This influence of C on denitrification is both through the provision of C directly to the denitrifiers, and/or stimulation of microbial metabolism, which increases the consumption of $O_2$ and creates conditions favorable for denitrification (Beauchamp et al., 1989). Laboratory studies have been conducted using simple C substrates (Weier et al., 1993; Jarvis and Hatch, 1994); however, fewer studies have used complex C sources that are common inputs in agricultural systems, such as crop residues (Dendooven et al., 1996). This is primarily due to the difficulty in assessing C availability in these more complex C sources (Beauchamp et al., 1989).

Microbial denitrification is believed to be a primary source of $N_2O$ (Wrage et al., 2004); however, few studies have examined the denitrifier community and their influence on $N_2O$ emissions. Denitrifier bacteria belong to a variety of physiological and taxonomic groups (Zumft, 1997) and have commonly been characterized by using most probable number (MPN) counts (Jacobson and Alexander, 1980; Lensi et al., 1995) and the denitrifier enzyme activity (DEA) assay (Martin et al., 1988). Using a modified DEA, Cavigelli and Robertson (2000) reported that under identical environmental conditions there was an increase in the $N_2O$ molar ratio in soil from an agricultural field compared with a successional field, and suggested there was a significant functional role of the denitrifier community. More recently, molecular methods have been used to examine the denitrifying community composition and diversity (Rich and Myrold, 2004; Boyle et al., 2006), as well as the abundance of denitrifiers (Henry et al., 2004, 2006; Kandeler et al., 2006; Dandie et al., 2007a) by focusing on the amplification of functional genes involved in denitrification. The genes include nitrate reductase (*napA* and *narG*), nitrite reductase (*nirS* and *nirK*), nitric oxide reductase (*qnorB* and *cnorB)*, and nitrous oxide reductase (*nosZ*). However, not all denitrifying bacteria produce the complete suite of enzymes required to complete the denitrification process (Zumft, 1997).

Literature reviews have suggested that the composition and density of soil denitrifier communities may be factors affecting denitrification (Philippot and Hallin, 2005; Wallenstein et al., 2006), with studies reporting that the denitrifier community differs in response to environmental conditions that indirectly control the rate of denitrification (Cavigelli and Robertson, 2000; Holtan-Hartwig et al., 2000). Therefore, there is a need to understand the community dynamics of denitrifiers and the environmental factors influencing the abundance of the denitrifiers in soil to determine if the denitrifying community may play a role in controlling denitrification. For example, few studies have evaluated how C sources will affect the denitrifier community, and more specifically, the abundance of bacteria possessing these functional genes. Studies using quantitative real-time PCR revealed that soil amended with a mixed C substrate resulted in a four-fold increase in the number of *nirK* gene copies as compared with the same soil amended with water (Henry et al., 2004). Although quantification of soil denitrifier gene copy numbers has been reported previously in ecological studies, few studies have analyzed the response of denitrifier community abundance in agricultural soils to C amendment treatments, and none of these studies have evaluated the influence of crop residues on the denitrifier community abundance while comparing it with denitrification activity measured using biochemical assays. The objective of this study was to determine the influence of crop residue amendments on: (i) the amount and $N_2O$ molar ratio of gaseous denitrification losses; and (ii) total bacterial and denitrifier community abundance. Bacterial abundance was measured using quantitative PCR and targeting the 16S rRNA gene for the total bacterial community, the *Pseudomonas mandelii* and related species $cnorB_P$, *Bosea*/*Bradyrhizobium*/*Ensifer* spp. $cnorB_B$ and *nosZ* functional genes for the denitrifier communities.

## 2. Materials and methods

### 2.1. Soil

In January 2006, frozen soil (0–15 cm) was collected from a field previously cropped to spring wheat (*Triticum aestivum* L.) in Fredericton, New Brunswick, Canada (45°52′ N, 66°31′ W). In order to maintain a low soil $NO_3^-$ concentration, the soil was kept frozen at −20 °C. Three days prior to experimentation, the soil was thawed, air-dried to a gravimetric water content of 0.30 g $g^{-1}$ dry weight, homogenized and passed though a 2 mm sieve and stored in the dark at 4 °C. Soil texture (pipette method with organic matter removal) was 406 g $kg^{-1}$ sand, 475 g $kg^{-1}$ silt and 119 g $kg^{-1}$ clay. Soil total N and organic C concentrations (LECO CNS-1000) were 1.72 g $kg^{-1}$ and 23.9 g $kg^{-1}$, respectively. Soil pH (1:1 water) was 6.2.

### 2.2. Experimental design

Two experiments were conducted using repacked soil cores, assembled as previously described by Gillam et al. (2008). Briefly, the appropriate quantity of each C amendment was thoroughly mixed into the soil. The soil was hand packed to a bulk density of 1 Mg $m^{-3}$ into Plexiglass cores. The cores were 5.5 cm in diameter by 6 cm high with a wall thickness of 0.5 cm, and had a series of 1 mm diameter holes to allow gas exchange. The soil cores were placed in 1 l canning jars and sealed with lids fitted with a perforable septum to allow for gas sampling. Each jar had either a 10% volume of acetylene ($C_2H_2$) or atmosphere added. The $C_2H_2$, generated from calcium carbide and water, was added to block $N_2O$ reduction to $N_2$. Each treatment core was packed at 4 °C and remained at this temperature for 12 h after $C_2H_2$ injection to limit microbial activity while $C_2H_2$ diffused throughout the soil core. The jars were then incubated at 25 °C for 144 h. Time zero was the time at which the jars were placed in the incubation chamber.

#### 2.2.1. Microcosm experiment 1

The objective of Experiment 1 was to determine the influence of C and $NO_3^-$ availability on the amount and $N_2O$ molar ratio of gaseous denitrification losses. The experiment used a factorial arrangement of treatments in a completely randomized design with twelve treatments replicated four times. The factors were three levels of C addition (0, 100 and 250 mg C $kg^{-1}$ dry soil as glucose) and four levels of $NO_3^-$ addition (0, 10, 25 and 50 mg N $kg^{-1}$ dry soil as $KNO_3$). Each treatment was added in solution to the soil to attain a water-filled pore space (WFPS) of 70%. Each treatment was incubated with and without the addition of $C_2H_2$. At the end of the experiment (144 h), soil cores were sub-sampled for determination of $NO_3^-$ and ammonium ($NH_4^+$) concentrations. Headspace gas samples (20 ml) were taken from each jar at 0, 8, 16, 24, 36, 48, 72, 96, 120 and 144 h and placed in an evacuated Exetainer vial (Labco, UK) containing 4 mg of magnesium perchlorate, a desiccant to remove water from the gas sample. An equal volume of air was injected into the jar immediately after sampling. Samples were analyzed for $N_2O$ and $CO_2$ by gas chromatography.

#### 2.2.2. Microcosm experiment 2

The objective of Experiment 2 was to determine the influence of crop residues on the amount and $N_2O$ molar ratio of gaseous denitrification losses and the abundance of the denitrifier community. The experiment used a completely randomized design with six treatments replicated four times. Each treatment was added to achieve a WFPS of 70%. The treatments included a control with no C addition and with addition of 50 mg $KNO_3$-N $kg^{-1}$ soil ($G_0N_{50}$), addition of 250 mg C $kg^{-1}$ soil as glucose without or with addition of 50 mg $KNO_3$-N $kg^{-1}$ soil ($G_{250}N_0$ and