Glucose effects on denitrifier abundance, denitrification gene mRNA levels, and denitrification activity in an anoxic soil microcosm

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Abstract

Organic carbon availability influences nitrous oxide (N\textsubscript{2}O) emissions but its effect on denitrifier communities is not understood. Changes in denitrifier abundance, denitrification gene mRNA levels and denitrification activity were followed in anoxic soil microcosms in the presence and absence of glucose with non-limiting nitrate concentration for 48 h. \textit{nosZ} and \textit{nirS} (\textit{Pseudomonas mandelii} and closely-related spp.) genes (qPCR) and mRNA levels (qRT-PCR) were quantified. Abundance of \textit{nosZ} and \textit{nirS} were unaffected by glucose addition and were stable over the duration of the incubation with average values of 4.3 \times 10^8 and 8.1 \times 10^8 gene number/g dry soil, respectively. \textit{nirS} mRNA levels were increased by glucose addition. Glucose addition resulted in induction of \textit{nirS} mRNA levels after 4 h, with a 2.5 fold increase in transcripts compared with 0 h, to 2.4 \times 10^8 transcripts/g dry soil. In contrast, \textit{nosZ} mRNA levels were not affected by glucose addition and averaged 2.3 \times 10^8 transcripts/g dry soil. Glucose addition increased cumulative N\textsubscript{2}O emissions, with final values of 4.9 and 0.9 mg N\textsubscript{2}O-N/kg dry soil for the glucose amended and non-amended soils, respectively, at 48 h. The increase in N\textsubscript{2}O emissions resulting from glucose addition in this study were not clearly accompanied by significant changes in abundance or denitrification gene mRNA levels for the targeted bacterial communities.

Key Words

Introduction

Denitrification, the dissimilatory reduction of nitrogen oxides, is a metabolic process performed by soil bacteria that produces the greenhouse gas nitrous oxide (N\textsubscript{2}O) as an intermediate gaseous product. Denitrification is influenced by environmental factors including oxygen concentration and nitrate concentrations; however, carbon availability is likely one of the most important factors influencing denitrification (Miller \textit{et al.} 2008). Organic carbon addition reduces soil oxygen supply by promoting microbial growth, favouring the denitrification process. Organic carbon is also used as an electron donor in denitrification (Zumft 1997). Glucose, a simple carbon source, was used in several denitrification studies (Dandie \textit{et al.} 2007, Fischer \textit{et al.} 2005, Miller \textit{et al.} 2008, Murray \textit{et al.} 2004) and was used in this study as a first step in a larger project to understand the relationships between denitrifier activity and organic carbon sources in agricultural soils. The objective of this study was to evaluate the effect of a simple carbon source, glucose, on denitrifier abundance, denitrification gene mRNA levels, and denitrification activity from an agricultural soil. We hypothesized that an increase in denitrification activity, after the addition of glucose, would be due to an increase in the abundance of denitrifiers and/or the abundance of denitrification gene transcripts.

Methods

Experimental Design

Treatments with or without glucose addition (0 or 500 mg glucose-C/kg dry soil) were applied to soil cores in a factorial arrangement (2 levels of glucose, 6 incubation times, 4 replicates) in a completely randomised design. Nitrate was added at 500 mg NO\textsubscript{3}-N/kg dry soil (as KNO\textsubscript{3}) and all cores were incubated at 70\% water-filled pore space in sealed jars. Two sets of jars were used to measure denitrification and N\textsubscript{2}O emissions. Cumulative denitrification was measured in one set of jars by adding acetylene (C\textsubscript{2}H\textsubscript{2}) to the headspace to block N\textsubscript{2}O reduction (N\textsubscript{2}O + N\textsubscript{2}). No C\textsubscript{2}H\textsubscript{2} was added to a second set of jars to measure N\textsubscript{2}O emissions. Headspace gas samples (20 mL) were taken using a syringe. Soils were destructively sampled at...
0, 4, 8, 12, 24, and 48 h of incubation. For nucleic acid extractions, soil samples were flash frozen in liquid nitrogen immediately after sampling and stored at -80°C until processing.

**Analyses and statistics**

Extractable organic carbon (EOC) and NO₃⁻ concentrations were measured in K₂SO₄ extracts from soil samples using colorimetric analysis on a Technicon Auto Analyzer II system (Technicon Industrial Systems, Terrytown, MA, USA). Headspace gas was analyzed for N₂O and CO₂ concentrations using a Varian Star 3800 Gas Chromatograph (Varian, Walnut Creek, CA) fitted with an electron capture detector (to measure N₂O), thermal conductivity detector (to measure CO₂), and a Combi-PAL Autosampler (CTC Analytics, Zwingen, Switzerland) (Burton et al. 2008). Nucleic acids (DNA and RNA) were extracted from freeze-dried soil samples using methods adapted from Griffiths et al. (2000).

Denitrification genes and transcripts (nosZ and nirS) were quantified via qPCR and qRT-PCR using an Applied Biosystems (Streetsville, ON) ABI PRISM® 7000 thermal cycler and SYBR Green detection as described in Henderson et al. 2010. ANOVA was performed with treatment and time as fixed factors. Means comparisons were performed for significant main effects and interactions by performing post hoc Tukey HSD and Tukey adjusted LS means, respectively.

**Results and discussion**

![Graph A](image1.png)

**Figure 1.** Soil concentrations of nitrate (NO₃⁻) (A) and extractable organic carbon (EOC) (B) for soil incubated over 48 h following addition of glucose at 0 mg C-glucose/kg dry soil (G0) (□) or 500 mg C-glucose/kg dry soil (G500) (■). Values are means ± SEM (n=4).

![Graph B](image2.png)

**Figure 2.** Cumulative emissions of nitrous oxide (N₂O) and denitrification (i.e. N₂O + N₂) (A) and carbon dioxide (CO₂) (respiration) (B) from soil incubated over 48 h following addition of glucose at 0 mg C-glucose/kg dry soil (G0) with (△) or without (□) C₃H₈, or addition of 500 mg C-glucose/kg dry soil (G500) with (■) or without (□) C₃H₈. Values are means ± SEM (n=4).
Effect of glucose on analytical measurements

Glucose addition resulted in a significant (p = 0.048) decrease in soil NO$_3^-$ concentration, with average values of 425 and 373 mg NO$_3^-$-N/kg dry soil in the G0 and G500 treatments, respectively (Figure 1A). The EOC concentration significantly (p < 0.001) increased following glucose addition with average values of 37 and 367 mg C/kg dry soil in the G0 and G500 treatments, respectively (Figure 1B). Reduction in EOC concentration over time indicated that glucose was metabolized.

Over the 48h incubation period, there was no significant difference between cumulative N$_2$O production from soil incubated without C$_2$H$_2$ (i.e. N$_2$O emissions) or with C$_2$H$_2$ (i.e. total denitrification) (Figure 2A), indicating that gaseous emissions from denitrification occurred primarily as N$_2$O. Cumulative denitrification was significantly (p = 0.002) increased by glucose addition (Figure 2A). Addition of glucose significantly increased respiration (cumulative CO$_2$ emissions) (Figure 2B). Similarly, soil amendment with glucose and other sources of organic carbon such as plant residues or manure have previously been shown to increase denitrification activity, and the increase in denitrification was commonly related to the increase in respiration (Dandie et al. 2007; deCatanzaro et al. 1985; Miller et al. 2008; Miller et al. 2009).
**Molecular analysis of denitrifier abundance and mRNA levels**

Despite the increases in respiration and denitrification in response to glucose addition, there were no measurable changes in the nir\textsubscript{S}p (Figure 3A) and nosZ (Figure 4A) abundance in soil over the 48 h incubation period. Previous studies also found that the nosZ-bearing denitrifier community did not increase in abundance after the addition of glucose to anoxic soil microcosms in 6 day incubations (Miller et al. 2008). In contrast, glucose addition at 250 mg-C /kg dry soil increased abundance of \textit{P. mandelii} and closely related species quantified using \textit{cnorB} primers (\textit{cnorB}\textsubscript{p}) in anoxic soil microcosms (Dandie et al. 2007, Miller et al. 2008). In soil amended with glucose, nir\textsubscript{S}p gene transcript abundance was increased compared with unamended soil only at 4 h (\(p = 0.009\)) (Figure 3B). The increase in nir\textsubscript{S}p transcripts occurred without a measurable increase in nir\textsubscript{S}p abundance, suggesting this increase was through increased mRNA levels per cell. Surprisingly, nosZ mRNA levels were not significantly affected by glucose addition and did not change significantly over time during the 48 h incubation (\(p = 0.320\)) (Figure 4B). Glucose is commonly thought to induce denitrification gene expression through oxygen depletion resulting from increased microbial respiration, however in this experiment where soil anoxic conditions were implemented by changing the headspace gas composition, such a response would not occur.

We hypothesized that the measured denitrification increase in glucose amended soil was due to an increase in denitrifier abundance and/or denitrification gene mRNA levels. Soil amendment with glucose increased microbial respiration and denitrification without a significant increase in abundance of nosZ and nir\textsubscript{S}p denitrifier communities and with a measurable and transient increase in transcripts for nir\textsubscript{S}p only. Therefore, under the experimental conditions used, the increase in denitrification activity was not well linked to denitrifier gene copy and mRNA suggesting that enzyme activity might be important in understanding the control of N\textsubscript{2}O emissions in soil systems.

**References**


