Soil Biology & Biochemistry 40 (2008) 2796–2802

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Is nitrate reduction to nitrite possible in glucose-amended alkaline saline soil under aerobic conditions?

C. Vega-Jarquin, C. Valenzuela-Encinas, I. Neria-González, R.J. Alcántara-Hernández, M.A. Hernández-Santiago, M.L. Luna-Guido, R. Marsch, L. Dendooven*

Laboratory of Soil Ecology, Department of Biotechnology and Bioengineering, Cinvestav, Av. Instituto Politécnico Nacional 2508, C.P. 07000 México D.F., Mexico

ARTICLE INFO

Article history:
Received 18 January 2008
Received in revised form 24 July 2008
Accepted 31 July 2008
Available online 27 August 2008

Keywords:
Aerobic incubation experiment
Assimilatory reduction
Concentrations of ammonium, nitrite and nitrate
Emissions of CO_2
Glucose

ABSTRACT

A phylogenetic analysis of the archaeal community in the soil of the former Lake Texcoco showed that some of the clones identified were affiliated to Archeae that reduce nitrate (NO_3^-) to nitrite (NO_2^-) and NO_2^- to unknown products under aerobic conditions. Previous research suggested that this indeed might occur when an easily decomposable C-substrate is available, but little is known about the factors that control the possible processes involved. The sandy clay loam soil with pH 10 and electrolytic conductivity 56 dS m^{-1} was spiked with 1000 mg glucose-C kg^{-1} soil (GLUCOSE pre-treatment), 200 mg NO_3^--N kg^{-1} soil (NITRATE pre-treatment), or left unamended (CONTROL pre-treatment) and conditioned for eight days. Pre-treated soil was then added with 1000 mg glucose-C kg^{-1} soil and 200 mg NO_3^--N kg^{-1} soil and amended with ammonium (NH_4^+) (AMM treatment) and L-glutamine (GLUT treatment), acetylene (C_2H_2) (ACE treatment), oxygen (O_2) (OXI treatment), left untreated (CON treatment) or sterilized. No abiotic factors affected concentrations of NH_4^+, NO_2^- or NO_3^-. In the CONTROL pre-treatment, concentration of NO_3^- decreased 170 mg N kg^{-1} soil within 72 h, in the GLUCOSE pre-treatment with 182 mg N kg^{-1} soil within 2 h and in the NITRATE pre-treatment with 272 mg N kg^{-1} soil within 168 h. Mean concentration of NO_2^- was 3.2 mg N kg^{-1} soil in unamended soil, 5.7 mg N kg^{-1} soil in the CONTROL pre-treatment, but >20 mg kg^{-1} soil in the GLUCOSE pre-treatment and ≥40 mg kg^{-1} in the NITRATE pre-treatment. The application of NO_3^- and glucose increased the mean concentration of NH_4^+ compared to the unamended soil independently of pre-treatment. It was found that microorganisms in the alkaline saline soil of the former Lake Texcoco can reduce concentrations of NO_3^- while releasing NO_2^- under aerobic conditions when an easy decomposable substrate is available without it being directly related to microbial activity and this being more outspoken when glucose or nitrate were previously added.

1. Introduction

Soil of the former Lake Texcoco somewhat to the east of Mexico City is extreme alkaline saline with pH locally more than 11 and electrolytic conductivity (EC) more than 80 dS m^{-1} (Luna-Guido et al., 2000). Not much information is available about this kind of soils and only a few studies have been published investigating processes related to the N cycle in sediments of salt lakes (Sorokin et al., 2001). A phylogenetic analysis of the archaeal community in the soil of the former Lake Texcoco showed that some of the clones identified were closely related (95.21–96.00% similarity) to the genera *Natrinema* (Valenzuela-Encinas et al., 2008). *Natrinema pallidum* and *Natrinema pellirubrum*, isolated from salted fish and hides (Formisano, 1962), are strictly aerobic, can use glucose as C source and reduces nitrates to nitrite and to unknown end products (McGenity et al., 1998). Another clone was affiliated to an extreme halophilic archaeon *Natronococcus amylolyticus* (Kanai et al., 1995). It is also strictly aerobic and can reduce nitrate and nitrite. Clones affiliated to *Halorubrum alkaliphilum* sp. nov. were also found and they did not grow under anaerobic conditions with nitrate, but reduction of nitrate to nitrite was observed under aerobic conditions (Feng et al., 2005).

Addition of 200 mg nitrate-N (NO_3^--N) kg^{-1} and an easily decomposable substrate, such as maize, biosolid or glucose, to soil of the former Lake Texcoco induced a sharp decrease in the concentration of NO_3^- under aerobic conditions with a sharp increase in the concentration of nitrite (NO_2^-), but no such increase was found in sterilized or unamended soil (Beltrán-Hernández et al., 1999; Luna-Guido et al., 2001; Vega-Jarquin et al., 2003; Conde et al., 2005; Dendooven et al., 2006). Considering the fact that the above mentioned Archaea found in soil of Texcoco can reduce NO_3^- to NO_2^- under strict aerobic conditions and the evidence from previous

* Corresponding author. Tel.: +52 5560513311; fax: +52 5560513313.
E-mail address: dendoove@cinvestav.mx (L. Dendooven).

0038-0717/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2008.07.028

experiments that NO_3^- disappeared while NO_2^- was formed under aerobic conditions suggest that Archeae might be involved in this yet not fully understood process of reduction of NO_3^- under aerobic conditions. As part of a study into the factors that might affect the reduction of NO_3^- under aerobic conditions (i) soil of Texcoco was conditioned for seven days, (ii) amended with 1000 mg glucose-C kg^{-1} and conditioned for seven days so that concentrations of inorganic N became low, i.e. N immobilization or (iii) amended with NO_3^- and conditioned for seven days so that an excess of inorganic N was found in soil. In a second step, processes that might affect concentrations of NO_3^- or NO_2^- in an aerobic incubation experiment were inhibited. First, denitrification was inhibited by incubating soil under 100% oxygen (O_2) as it represses all anaerobic respiratory pathways and fermentation in soil (Robertson and Tiedje, 1987). Second, autotrophic nitrification, which might contribute to an increase in concentrations of NO_2^- and NO_3^-, was inhibited by adding 0.1% (v/v) acetylene (C_2H_2) to the headspace (Wrage et al., 2001). Third, assimilatory nitrate reductase was inhibited by adding L-glutamine or NH_4^+ to soil (e.g. Rice and Tiedje, 1989). Fourth, soil was sterilized to investigate possible abiotic processes that might affect concentrations of NO_2^- and NO_3^-. In a third step production of CO_2 and concentrations of NH_4^+, NO_2^- and NO_3^- were monitored in an aerobic incubation experiment. The objective of this study was to investigate factors that affect concentrations of NO_3^- and NO_2^- in an alkaline saline soil of the former Lake Texcoco under aerobic conditions and processes that might be involved when an easily decomposable C-substrate is available.

2. Materials and methods

2.1. Experimental site, collection and characteristics of the soil

The soil of the former Lake Texcoco in the valley of Mexico City (Mexico) at an altitude of 2240 m above sea level with a mean annual temperature of 16 °C and annual precipitation of 705 mm is formed from volcanic ash deposited *in situ* in a lacustrine environment and covered recently by colluvial materials. The aquifer is near to the surface (80–150 cm) and the groundwater is highly saline, sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) being dominant. pH in undrained soil can range from 9.8 to 11.7, electrolytic conductivity (EC) in saturation extracts from 22 to 150 dS m^{-1}, exchangeable sodium percentages from 76 to 98% and sodium adsorption ratio from 103 to 1718 mmol l^{-1} (Luna-Guido et al., 2000).

The mineralogy is dominated by amorphous siliceous materials, smectites, kaolinite and cristobalite (Gutierrez-Castorena et al., 2005). Drainage of the area has created cracks 1 m deep and 10 cm wide. The amorphous material increases phosphorus (P) fixation; retention is especially high in the moist subsoil (80%), in the topsoil values range from 26 to 31% (INEGI, 1994).

Parts of the former lake bed have been drained and irrigated with sewage effluent from a wastewater plant to remove excess of salt. *Distichlis spicata*, an indigenous grass with a high tolerance to salt and Na^+, and tamarix (*Tamarix* spp.) have been introduced since the early 1970s to control erosion, and they now cover much of the area. More details on the experimental site, the vegetation and the effluents used to drain the plots can be found in Luna-Guido et al. (2000).

Soil was sampled on 11 March 2002 at random by augering 25-times (auger ∅7 cm, Eijkelkamp, Nl) from the 0–10 cm layer of three different plots with a size of ca 400 m^2 in a 10 ha field. The sandy clay loam soil with pH 10 and EC 56 dS m^{-1}, had an organic carbon (C) content of 53 g C kg^{-1} soil, an inorganic C content of 11 g C kg^{-1} soil, a total N content of 6.7 g N kg^{-1} soil, a water holding capacity (WHC) 740 g kg^{-1} and a particle distribution of 220 g clay kg^{-1} soil, 180 g silt kg^{-1} soil and 570 g sand kg^{-1} soil.

2.2. Treatments and experimental set-up

The soil samples were taken to the laboratory and were treated as follows. The soil from each plot, approximately 12 kg, was separately passed through a 5 mm sieve, adjusted to 40% WHC by adding distilled H_2O and pre-incubated for seven days in a drum containing a beaker with 100 ml distilled H_2O to avoid desiccation and one beaker with 100 ml of 1 M NaOH solution to trap CO_2 evolved. From each plot, 270 sub-samples of 20 g soil were added to 120 ml glass flasks.

The experimental plan was complex (Fig. 1) as it involved three different pre-treatments and five treatments. In a first step, 75 sub-samples of 20 g soil from each plot were amended with 1000 mg glucose-C kg^{-1} soil (GLUCOSE pre-treatment), 75 with 200 mg NO_3^--N kg^{-1} soil (NITRATE pre-treatment) and 120 served as control (CONTROL pre-treatment). Those three pre-treatments were applied to have an N-depleted soil (GLUCOSE pre-treatment), a soil with excess of N (NITRATE pre-treatment) and a control soil (CONTROL pre-treatment). The soils were conditioned for eight days.

In a second step, 75 sub-samples of soil of each pre-treatment were amended with 200 mg KNO_3-N kg^{-1} soil and 1000 mg glucose-C kg^{-1} soil and five treatments were applied. Fifteen sub-samples were added with 200 mg $(NH_4)_2SO_4$-N kg^{-1} soil (considered the AMM treatment), 15 with 200 mg L-glutamine-N kg^{-1} soil (considered the GLUT treatment), 15 kept under 100% O_2 atmosphere (v/v) (considered the OXI treatment), 15 kept under a 0.1% C_2H_2 (v/v) (considered the ACE treatment) and 15 were left unamended (considered the CON treatment). NH_4^+ and L-glutamine were added to soil because they are known to inhibit assimilatory reduction of NO_3^- (e.g. McCarty and Bremner, 1992; Rice and Tiedje, 1989), C_2H_2 is known to inhibit autotrophic nitrification (e.g. Wrage et al., 2001) while O_2 is known to inhibit anaerobic processes (e.g. Schirawski and Unden, 1995). The amount of solution added to the soil resulted in a water content of 50% WHC, sufficient for normal microbial activity, but low enough to avoid anaerobicity. Additionally, 30 sub-samples of the CONTROL pre-treatment were sterilized three times for 30 min with an interval of a day with pressurized steam at 121 °C supplied by an autoclave (Wolf and Skipper, 1994). Fifteen of the sterilized samples were amended with 200 mg $(NH_4)_2SO_4$-N kg^{-1} soil plus 200 mg KNO_3-N kg^{-1} soil under sterile conditions while 15 with 200 mg L-glutamine-N kg^{-1} soil. Sterilization of the soil allowed to distinguish biotic from abiotic processes that might affect concentrations of NH_4^+, NO_2^- and NO_3^-. The remaining 15 sub-samples of the CONTROL pre-treatment were only amended with distilled H_2O to 50% of WHC and served as controls.

In a third step, three flasks were chosen at random from each treatment. The soil was extracted for inorganic N with 120 ml 0.5 M K_2SO_4 solution to provide zero-time samples. The samples were shaken for 60 min and filtered through Whatman No 42 paper® and stored pending analysis at −20 °C. The remaining glass flasks were placed in 945 ml glass jars containing a vessel with 10 ml distilled H_2O and one with 20 ml 1 M NaOH. The jars were sealed and stored in the dark at 22 ± 2 °C. An additional 12 jars containing a vessel with 10 ml distilled H_2O and one with 20 ml 1 M NaOH were sealed and served as controls to account for the CO_2 trapped from the air. After 6, 24, 72 and 168 h, three jars were selected at random from each treatment, opened, and the vessel containing NaOH removed and stoppered pending analysis. The soil was removed from three flasks and extracted for inorganic N as described before.

2.3. Soil chemical analysis

Soil pH was measured in 1:2.5 soil–H_2O suspension using a glass electrode (Thomas, 1996). Large concentrations of Cl^- might affect the estimation of C by wet oxidation (Nelson and Sommers, 1996)