Nitrification in polluted soil fertilized with fast- and slow-releasing nitrogen: A case study at a refinery landfarming site

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Nitrification occurs in soil containing high concentration of aged oil.

Abstract

The nitrifying activity and the effect of fertilization with urea and methylene urea were studied in a landfarming site. The site has been operative over 20 years and maintained by heavy nitrogen fertilization. The landfarming soil contained 4–6% (w/w) oil. The nitrate accumulation was 20–50 mg NO_3-N day^{-1} kg^{-1} observed after methylene urea fertilization of 889 g N m^{-2}. Nitrification ex situ (in laboratory conditions) was 8.8 mg NO_3-N day^{-1} kg^{-1} in the presence of 380 mg kg^{-1} NH_4^+-N. The half-saturation concentration of nitrification was more than 200 mg NH_4^+-N kg^{-1}. The results show that nitrification was active in soil with high oil concentration. Urea fertilization of 893 g N m^{-2} caused an increase of soil NH_4^+-N concentration up to 5500 mg kg^{-1} and pH > 8.5. This led to inhibition of nitrification, which persisted after NH_4^+ concentration decreased below 200 mg NH_4^+ kg^{-1}.
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1. Introduction

Oil industry generates large volumes of oily sludges creating a disposal problem. A widely used treatment method for oily wastes, especially sludges, is to blend the waste with soil and rely on the catabolic activities of the indigenous microbiota (Dibble and Bartha, 1979; Genouw et al., 1994; Alexander, 1999; Giasi and Morelli, 2003; Liu et al., 2004). Tilling and fertilizing, similarly to the agricultural practice, are used as tools to increase activity of the degrading soil microbiota. This bioremediation procedure is referred to as landfarming. Tilling and adding bulking agents improve oxygen transport in soil, irrigation ensures humidity for degrader microbes (Giasi and Morelli, 2003). As mineral oil addition increases the soil carbon content, amending the landfarming soil with nitrogen and phosphorus is needed to create a nutrient balance favourable for the degrading microbiota (Alexander, 1999). Widely varying C:N:P ratios have been applied in bioremediation practises (Beaudin et al., 1999; Liu et al., 2004). To ensure nutrient availability the landfarming soil is often overfertilized. (Alexander, 1999). This may compromise the treatment as the enhancing effect of nutrients on oil degradation has been observed to diminish at the highest levels of fertilization (Dibble and Bartha, 1979; Genouw et al., 1994).

Organic and inorganic pollutants have been observed to inhibit nitrification in soil, disturbing the soil nitrogen cycle (Miller et al., 1997; Gallardo and Merino, 1998; Lindstrom

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et al., 1999; Deni and Penninckx, 1999; Smolders et al., 2001; Cela and Sumner, 2002). There is ample data on nitrogen transformations in heavy metal and pesticide stressed soils (Giller et al., 1998; Malkomes, 2001; Ahtiainen et al., 2003). Oil-contaminated soil has received less attention, especially soil with a long history of oil pollution. Understanding nitrogen cycling is needed for success in treatment of soil polluted with oil waste, but published information on this issue is limited.

We used the landfarming field of an oil refinery as the experimental ground to assess tolerance of nitrifying bacteria to environmental stress. The field had been receiving oily sludges from the refinery for more than 20 years and had been heavily fertilized with urea (900 g urea-N m^{-2} per year). Experimental plots were fertilized with different amounts of urea and a slow-release form of nitrogen, methylene urea. High nitrification activity was found, characterized by high tolerance and low affinity to NH_4^+. High loads of urea-N caused an inhibition of the nitrification activity.

2. Materials and methods

2.1. Field test design

An oil-waste landfarming field that had been in operation for over 20 years in southern Finland (60°15′ N, 25°30′ E) was used as the test field. The land-farming field had received oily sludges from a refinery, the latest application two months before the start of the experiments. High applications of urea (900 g urea-N m^{-2} per year) had routinely been added to the field as a nitrogen fertilizer. The soil had been amended with wood chips one year before the study to improve porosity of the soil. The oil concentration of the soil varied from 35 to 59 g kg^{-1} dry wt. (Table 1). Loss of ignition of the soil was 20.3%. The test plots were 10 m wide and length varied from 34 to 54 m because of the irregular shape of the test field. Each plot (343–544 m^2) was fertilized with methylene urea (Duralene-P 38 N® medium, Kemira GrowHow Oy, Helsinki, Finland) or with urea (Kemira GrowHow Oy) on 8 August as described in Table 1. All test plots were equally amended with superphosphate 85 g m^{-2} (Kemira GrowHow Oy). Dolomite lime was added 73 g m^{-2} (Kemira Grow-How Oy). After the amendments the test field was ploughed to a depth of 20 cm. The plots were sampled five times, including pre-treatment. Triplicate samples were collected as 20 cm cores and were composited at each sampling time.

2.2. Analytical protocols

The oil in the landfarming soil was strongly weathered and thus not suitable for gas chromatographic analysis. Therefore the soil concentrations of oil

were measured gravimetrically (Dibble and Bartha, 1979; Genouw et al., 1994; Beaudin et al., 1999). The 5 g samples (two parallels from each sample) were extracted three times with 15 ml of dichloromethane (Sigma, St. Louis, MO, USA). The non-polar fraction of the extract was separated by passing the dichloromethane extract through a column of aluminium(III) oxide (Al_2O_3, Merck, Darmstadt, Germany). The eluate was evaporated and the obtained non-polar residue (mineral oil) was weighed.

Soil pH was measured from 5 g of soil in 25 ml 0.01 M $CaCl_2$ after 2 h/ 200 rpm in a rotary shaker.

Nitrate- and ammonium nitrogen was measured by extracting 100 g soil sample with 100 ml 2 M KCl. Nitrate in the extract was reduced to nitrite in alkaline conditions where copper catalyses the reaction. Nitrite determination was done according to colorimetric diazo complex method (Greenberg et al., 1980). Ammonium nitrogen determinations were done according to colorimetric salicylate complex method (Krom, 1980). The colorimetric determinations were done with a TRAACS 800 auto analyser (Bran and Luebbe, Norderstedt, Germany).

Nitrifying activity ex situ was measured by incubating 5 g of field moist soil (two parallels of each) in 20 ml vials with 240 mg NH_4^+ kg^{-1} (equalling 187 mg NH_4^+-N) as substrate, added as $(NH_4)_2SO_4$. The samples were incubated for 21 days at 25 °C. Nitrate was reduced to nitrite and measured at 210 nm with LKB Biochrom 4060 UV/VIS spectrophotometer (Amersham Biosciences, Little Chalfont, England) as described by Alef (1995).

Heterotrophic plate count was measured by extracting 10 g soil sample (two parallels from each sample) with 90 ml extraction solution (0.9% NaCl, 0.116% $Na_6O_{18}P_6$, 0.0022% Tween-80). The extractants were plated on 1:5 diluted plate count agar (Anonymous, 1998) and incubated at room temperature.

3. Results

Nitrogen transformation response to urea and methylene urea was investigated in soil contaminated with oils from refinery waste for over 20 years. The soil had more than 10 years history of nitrogen fertilization with urea targeted to give a carbon: nitrogen ratio of 10:1 to 10:2. At the time of the research the soil contained 35–59 g kg^{-1} non-polar solvent extractable organic matter (mineral oil) (Table 1).

Seven test plots on the landfarming field were fertilized with 0–890 g N m^{-2} (Table 1) of methylene urea or urea. The NO_3-N content of the fertilized test plots transiently increased by 400–800 mg kg^{-1} (Fig. 1). In addition, a part of the NO_3 formed by nitrification may have been lost from the soil by leaching, denitrification and immobilization into microbial biomass. The highest increase of nitrate was observed in the plot E fertilized with highest dose of methylene urea (889 g N m^{-2}). There was also nitrate accumulation

Table 1
Treatment characteristics and concentration of mineral oil in the test plots

Plot code	Area (m^2)	Soil density (kg dm^{-3})	Mineral oil concentration (g kg^{-1} dry wt)	Nitrogen fertilization		$C_{mineral\ oil}$:$N_{fertilizer}$
			mean ± SD	Fertilizing with	Amount (g N m^{-2})	
A	470	0.78	59 ± 6.70	None		
B	543	0.79	47 ± 5.15	Methylene urea	17	100:0.3
C	544	0.77	48 ± 11.3	Methylene urea	100	100:1.7
D	460	0.75	47 ± 6.30	Methylene urea	200	100:3.5
E	511	0.86	42 ± 5.12	Methylene urea	889	100:15
F	343	0.81	41 ± 3.25	Urea	100	100:1.9
G	468	0.84	35 ± 6.9	Urea	893	100:19

The $C_{mineral\ oil}$:$N_{fertilizer}$ ratios are calculated from the soil densities, working depth (20 cm) and the average C-content of mineral oil (80%).