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Mediating N_2O emissions from municipal solid waste landfills: Impacts of landfill operating conditions on community structure of ammonia-oxidizing bacteria in cover soils

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ABSTRACT

Chemolithotrophic ammonia-oxidizing bacteria (AOB) can produce N_2O, a highly potent greenhouse gas. Denaturing gradient gel electrophoresis (DGGE) analyses of the ammonia monooxygenase structural gene (*amoA*) and 16S rDNA gene were used to investigate the AOB community structure in the cover soils of municipal solid waste (MSW) landfills under three operating conditions: (a) MSW with soil cover, (b) MSW with soil cover, irrigation piping and vegetation, and (c) MSW covered with high-density polyethylene (HDPE) liner, soil cover, irrigation piping and vegetation. AOB species in MSW cover soils were significantly distinguished by the operation of HDPE liner isolation. The community structures of the *Nitrosomonas europaea*-like AOB species dominated in soils without HDPE liner isolation, whether vegetation and irrigation with landfill leachate existed or not, whereas *Nitrospira*-like AOB species dominated in soils with HDPE liner isolation. Lower N_2O flux from the soils with HDPE liner isolation would be partially related to these special community structures.

1. Introduction

Chemolithotrophic ammonia-oxidizing bacteria (AOB) are responsible for the first, rate-limiting step in nitrification, and play a critical role in natural nitrogen cycling (Oved et al., 2001; Dong and Sun, 2007; Huang et al., 2007). Additionally, AOB can produce nitrous oxide (N_2O), a highly potent greenhouse gas, which also contributes to the deletion of ozone layer (Shaw et al., 2006). Natural and agricultural soils have been estimated to contribute 57% to the total global N_2O budget (Mosier et al., 1998; Kroeze et al., 1999). AOB community structure has recently been investigated in different soils, such as agriculture soil, meadow soil and forest soil (Avrahami et al., 2003; Mintie et al., 2003; Avrahami and Conrad, 2004). However, their community structure in MSW cover soil has not been reported yet. In fact, municipal solid waste (MSW) landfills are potentially high emitters of N_2O (Rinne et al., 2005). Understanding of AOB community structure in MSW cover soil will be favorable for attenuating greenhouse gas from landfills.

Based on 16S rDNA analysis of existing cultures, aerobic ammonia-oxidizing bacteria comprise two monophyletic groups. One group belongs to γ-Proteobacteria, including *Nitrosococcus oceani* and *Nitrosococcus halophilus*. The other group belongs to β-Proteobacteria and includes two genera: *Nitrosomonas* and *Nitrosospira* (Bothe et al., 2000). The majority of Proteobacteria AOB cluster within β-Proteobacteria, with the exception of a few marine γ-Proteobacteria strains (Mahmood et al., 2006). *Nitrosospira* species are frequently observed in soils. Specifically, *Nitrosospira* cluster 2 dominates in acid soils (Nugroho et al., 2005). *Nitrosospira* cluster 3 and 7 are the major AOB in tilled and fertilized soils, while *Nitrosospira* cluster 0 dominates under undisturbed and unfertilized grassland soil (Stephen et al., 1996; Bruns et al., 1999; Webster et al., 2002). *Nitrosomonas* species can also be predominant under the application of compost or secondary effluent of treated urban sewage to tilled soil (Kowalchuk et al., 1999; Oved et al., 2001). These data demonstrate that AOB community structure varies, responding to different environments.

Ammonia monooxygenase structural gene (*amoA*) encodes the α-subunit of ammonia monooxygenase (AMO), the key

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enzyme that catalyzes the oxidation of ammonia to hydroxylamine (Rotthauwe et al., 1997). It has been shown to be a useful molecular marker to study AOB in environmental system (Oved et al., 2001; Avrahami et al., 2003; Avrahami and Conrad, 2004). The phylogeny of *amoA* gene has been demonstrated to correspond largely to the phylogeny of the 16S rDNA gene (Purkhold et al., 2000). However, previous works also indicate that *amoA* gene is more specific (excluding all non-β-Proteobacteria AOB) but less sensitive (targeting all β-Proteobacteria AOB) than 16S rDNA gene (Rotthauwe et al., 1997; Purkhold et al., 2000; Nicolaisen and Ramsing, 2002).

Comparative analyses of *amoA*-based and 16S rDNA-based denaturing gradient gel electrophoresis (DGGE) were carried out to investigate β-Proteobacteria AOB community structure in MSW cover soils under three operating conditions: (a) MSW with cover soil, (b) MSW with cover soil, irrigation piping and vegetation, and (c) MSW covered with high-density polyethylene (HDPE), cover soil, irrigation piping and vegetation.

2. Materials and methods

2.1. Site description

The tested landfill sites, approximately 68,000 m^2, in Tianziling MSW Landfill, were located at a valley in the northern Hangzhou City (30°23′N, 120°12′E), eastern China. The operating conditions applied to MSW were illustrated in Fig. 1. Under condition A, the soil had directly covered the MSW for approximately 1.5 years and had not been irrigated or vegetated. Under condition B, the soil had directly covered the MSW for approximately 1 year and had been vegetated with *Festuca arundinacea Schreb* and *Nerium indicum*, and irrigated with landfill leachate. The irrigation pipes were installed 0.30 m below the surface. Under condition C, the soil was isolated from MSW by high-density polyethylene (HDPE) liner for approximately 1 year and had been irrigated with landfill leachate and vegetated with *F. arundinacea Schreb* or *N. indicum*. *F. arundinacea Schreb* was sowed as seeds. *N. indicum* was planted as seedling from the same tree nursery. HDPE is a polyethylene thermoplastic made from petroleum, resistant to many different solvents and has a wide variety of applications, including containers, plastics bags and piping systems, etc. HDPE is also used for cell liners in subtitle D sanitary landfills, wherein large sheets of HDPE are either extrusion or wedge welded to form a homogeneous chemical-resistant barrier, with the intention of preventing the pollution of soil and groundwater by the lecheate from solid waste. The soils for landfill cover were all obtained from neighborhood districts of the tested Tianziling MSW Landfill. The soils were stored together in warehouses and were randomly taken for covering MSW. Hence, the source of the soils used in different treatments could be regarded as the same. The difference in the microbial compositions of the tested soil samples could be attributed to different treatments for 1–1.5 years.

2.2. Soil sampling

The soil layer covering MSW was approximately 0.5 m deep. Soil samples were collected from the upper 10 cm of soil layer. The soil sample A1 was collected from the site under Condition A. The soil samples B1, B2 and B3 were collected from the sites under Condition B. The soil samples C1 and C2 were collected from the sites under Condition C. C1 was vegetated with *F. arundinacea Schreb*, and C2 was vegetated with *N. indicum*. Each sample was consisted of three subsamples, which were collected at a distance of approximately 10 m among each other at the same site, manually removed out stones and rhizome, and were then mixed to represent an even sample. The weight of each sample was about 2 kg.

Fresh soil samples were analyzed as soon as necessary, or air dried, sieved to <2 mm size and stored at 4 °C according to the pretreatment requirements for further physical and chemical analysis. Soil samples for molecular analysis were stored at −20 °C.

2.3. Physical and chemical analysis of soils

The physical and chemical characteristics of soils in this study are listed in Table 1. The soil moisture content was determined gravimetrically by drying at 105 °C for 24 h. pH was measured from fresh soil-water suspension (1:2.5 w/v). NH_4^+–N, NO_3^-–N and NO_2^-–N in soils were extracted by shaking 10 g fresh soil sample with 50 ml 2 mol l^{-1} KCl for 1 h before filtering and their concentrations were then analyzed using Standard Methods (Clesced et al., 1998). Total N (TN) was analyzed with the Kjeldahl method (Lu, 2000). Soil organic matter content was determined by organic carbon content multiplied by 1.732 (Lu, 2000). Soil particle size distribution was evaluated by the hydrometer method (Lu, 2000).

2.4. Statistical analysis

The statistical analysis utilized SPSS 12.0 software (SPSS, Inc. 2003. Chicago, IL, USA). The means for two groups of cases were compared by the independent-samples *t*-test procedure.

2.5. DNA extraction and PCR

2.5.1. DNA extraction

DNA extraction was modified from the reported method (Ye et al., 2007). 0.5 g of soil sample was mixed with 600 μl of extraction buffer (100 mmol l^{-1} Tris–HCl (pH 7.0), 100 mmol l^{-1} sodium EDTA (pH 8.0), 1.5 mol l^{-1} NaCl). 0.5 g of glass beads (0.1 mm diameter) were added and then blended in a Bead-beater (XW-80A, JINGKE, Shanghai, China) for 5 min. Afterwards, 60 μl of sodium

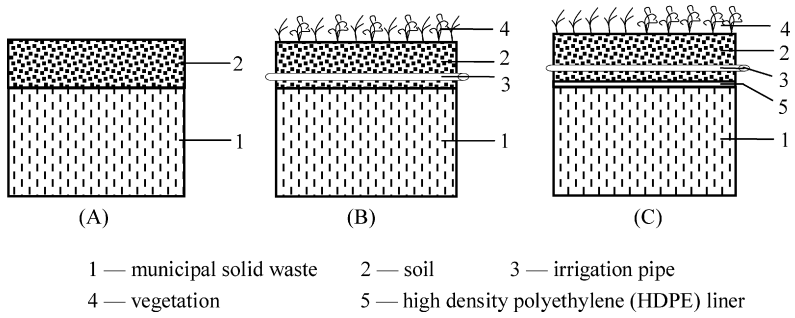

Fig. 1. MSW operating conditions. Condition A: MSW with soil cover. Condition B: MSW with soil cover, irrigation piping and vegetation. Condition C: MSW covered with high-density polyethylene (HDPE), soil cover, irrigation piping and vegetation.