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Effects of ammonium on the activity and community of methanotrophs in landfill biocover soils

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ABSTRACT

The influence of NH_4^+ on microbial CH_4 oxidation is still poorly understood in landfill cover soils. In this study, effects of NH_4^+ addition on the activity and community structure of methanotrophs were investigated in waste biocover soil (WBS) treated by a series of NH_4^+-N contents (0, 100, 300, 600 and 1200 mg kg^{-1}). The results showed that the addition of NH_4^+-N ranging from 100 to 300 mg kg^{-1} could stimulate CH_4 oxidation in the WBS samples at the first stage of activity, while the addition of an NH_4^+-N content of 600 mg kg^{-1} had an inhibitory effect on CH_4 oxidation in the first 4 days. The decrease of CH_4 oxidation rate observed in the last stage of activity could be caused by nitrogen limitation and/or exopolymeric substance accumulation. Type I methanotrophs *Methylocaldum* and *Methylobacter*, and type II methanotrophs (*Methylocystis* and *Methylosinus*) were abundant in the WBS samples. Of these, *Methylocaldum* was the main methanotroph in the original WBS. With incubation, a higher abundance of *Methylobacter* was observed in the treatments with NH_4^+-N contents greater than 300 mg kg^{-1}, which suggested that NH_4^+-N addition might lead to the dominance of *Methylobacter* in the WBS samples. Compared to type I methanotrophs, the abundance of type II methanotrophs *Methylocystis* and/or *Methylosinus* was lower in the original WBS sample. An increase in the abundance of *Methylocystis* and/or *Methylosinus* occurred in the last stage of activity, and was likely due to a nitrogen limitation condition. Redundancy analysis showed that NH_4^+-N and the C/N ratio had a significant influence on the methanotrophic community in the WBS sample.

Introduction

Aerobic CH_4 oxidation in oxic layers of landfill cover soils plays a significant role in reducing CH_4 emissions from landfills. CH_4 oxidation in these soils is affected by environmental factors such as soil texture, pH, soil moisture content, CH_4 and O_2 supply, nutrients and temperature [34]. Nitrogen is an important nutrient for microorganisms that can affect methanotrophic activities, and it subsequently interferes with the capacity of CH_4 oxidation in soil. Some studies have shown that higher NH_4^+ contents in soils tend to inhibit CH_4 oxidation, due to substrate competition between NH_3 and CH_4 for the active site of methane monooxygenase (MMO), which catalyzes the oxidation of CH_4 to methanol, and the toxicity of hydroxylamine and nitrite generated from NH_3 oxidation [23,24,37–39]. Different amounts of NH_4^+ might contradict the effects of CH_4 oxidation. The CH_4 oxidation rate in landfill cover soils was shown to decrease linearly with the initial NH_4^+ content of the soil when the NH_4^+-N content reached 25 mg-N kg^{-1} [4]. Similar results were obtained by Scheutz and Kjeldsen [33] who showed that CH_4 oxidation rates were unaltered when NH_4^+-N amended soil was 14 mg-N kg^{-1} or below, whereas the oxidation rates decreased at a higher NH_4^+-N content. Some studies, on the other hand, indicated that NH_4^+-N application could stimulate growth and activity of methanotrophs in landfill cover soils and rice fields [3,10,26,31]. When CH_4 flux is high enough to support the growth of methanotrophs, NH_4^+ can act more as a nutrient than as an inhibitor and strengthen the biological CH_4 sink [37]. The stimulative effect of NH_4^+ on CH_4 oxidation is also explained by the competition for nitrogen between the plant and the rhizosphere microbial community, thereby reducing the effective NH_4^+ concentration below a threshold that is inhibitory to methanotrophic activity [2]. In addition, since aerobic methanotrophs require oxygen for growth, sufficient oxygen generated from high plant density with nitrogen addition can stimulate CH_4 oxidation [2].

Aerobic methanotrophs are the major mediator in mitigating CH_4 emission from landfills, and most of them belong to *Proteobacteria* that can be classified into two major groups, type I and type

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II, based on their cell morphology, ultrastructure, phylogeny and metabolic pathways [14,36]. Nitrogen content has an important effect on the distribution of methanotrophs in the natural environment [34]. The activity and community of methanotrophs in a landfill cover soil with NH_4^+ addition has been hypothesized to occur in three stages, according to the growth and activity of methanotrophs with relatively high CH_4 mixing ratios (>1%) [9]. In the first stage, methanotrophs have a rapid growth rate, with higher rates probably obtained for type I methanotrophs. The second stage represents a decline in the methanotrophic activity, which might be caused by nitrogen limitation conditions for type I methanotrophs. In the third stage, a new growth phase can be observed that might be dominated by N_2-fixing type II methanotrophs. Compared to type I methanotrophs, type II methanotrophs can survive in a nitrogen-limited environment [12,22], which could be due to their nitrogen fixation capacity.

Biocover soils, such as compost, waste biocover soil (WBS) and mineralized waste, have high organic matter content, well-distributed particle size and active microorganism activity and have been demonstrated to be good alternative covers for mitigating CH_4 emission from landfills [5,17,33]. Ammonium is an important compound generated from the decomposition of deposited waste in landfills, especially in bioreactor landfills with leachate recycling [7,16]. The immediate effect of nitrogen (NH_4^+-N and NO_3^--N) addition on CH_4 oxidation and N_2O emission in biocover soils has been investigated for short periods (within 150 h) in batch experiments [42,44]. However, there is little information on the responses of methanotrophic communities and their activity to NH_4^+-N application in landfill biocover soils.

Therefore, the objective of this study was to test the effects of NH_4^+-N addition on the activity of the methanotroph community in landfill biocover soils. Batch experiments were conducted with a series of NH_4^+-N contents (0, 100, 300, 600 and 1200 mg kg^{-1}) in WBS. NH_4^+-N, NO_3^--N, total nitrogen (TN), total organic carbon (TOC) contents and the C/N ratio were determined during incubation. Quantitative PCR (Q-PCR), terminal restriction fragment length polymorphism (T-RFLP) and cloning of *pmoA* (encoding a subunit of the particulate MMO) were applied to analyze the identity and diversity of methanotrophs and their response to NH_4^+-N addition.

Materials and methods

Landfill cover soil microcosm

The WBS used in this study was taken from an organic waste landfill bioreactor (2 m^3) in a village located in Xindeng town, Zhejiang Province. After removing large particles, the soils were air-dried and sieved through a 4 mm mesh. The particle composition of the experimental WBS samples was 61% of 2–4 mm, 33% of 0.02–2 mm, 5% of 0.002–0.02 mm and 1% of <0.002 mm. The pH value was 7.7. The TN, NO_3^--N, NO_2^--N and NH_4^+-N contents of the WBS samples were 710.0 mg kg^{-1}, 674.3 mg kg^{-1}, 0.9 mg kg^{-1} and 33.4 mg kg^{-1}, respectively.

Approximately 70 g of the air-dried experimental WBS were placed into 400 mL serum bottles. The WBS was adjusted to a water content of 45% (w/w), at which the WBS was reported to have the highest CH_4 oxidation activity [42]. The NH_4Cl solution was added to the WBS samples by adding quantities of 100 mg-N kg^{-1}, 300 mg-N kg^{-1}, 600 mg-N kg^{-1} and 1200 mg-N kg^{-1} (denoted as WN100, WN300, WN600 and WN1200, respectively). The treatment without NH_4Cl addition was denoted as CK. The treatments with sterilized soils and NaN_3 (0.13 mg g^{-1} dry weight) were used as non-microbial controls to check if CH_4 disappearance occurred. All tests were performed in triplicate.

After the adjustment of the soil water and NH_4^+-N contents, the serum bottles were sealed with butyl rubber stoppers, and they were then covered with cling film and allowed to equilibrate overnight (~approximately 12 h) at 30 °C. A corresponding volume of air was taken from the serum bottles prior to injecting simulated landfill gas (LFG). Simulated LFG (CH_4:CO_2 = 1:1; v/v) was injected into the serum bottles at CH_4 and CO_2 concentrations of 10% (v/v). High purity O_2 was injected into the serum bottles at a concentration of ~21% (v/v). Gas samples were withdrawn periodically from the headspace of the serum bottles to measure CH_4, CO_2 and O_2 concentrations. The serum bottles were flushed with air and the initial concentrations of CH_4, CO_2 and O_2 were resupplied every 12 h to re-establish their initial concentrations. The whole experiment lasted for 56 days.

Soil sampling and analyses

Soil samples were withdrawn from the serum bottles after 7, 15, 30 and 56 days incubation. They were immediately used to measure soil water moisture, the contents of TN, NO_3^--N, NH_4^+-N, TOC and extracellular polymeric substances (EPS), including extracellular protein (ECP) and extracellular polysaccharide (ECPS), and the remaining samples were stored at −20 °C for subsequent molecular analysis. The subsample withdrawn at the end of the experiment was used to monitor soil pH value. The TN, NH_4^+-N, NO_3^--N and TOC contents were determined using the methods described by Bao [1]. The soil water content was measured by the loss of soil weight after drying to a constant weight at 105 °C.

EPS was measured using the method modified from McSwain et al. [29]. Approximately 2.0 g of fresh soil were placed into 50 mL centrifuge tubes containing 20 mL sterile water. After being centrifuged at 4 °C and 4000 rpm for 5 min, the supernatant was discarded and the clean soil sample was used to extract EPS using 15 mL of 1 M NaOH solution heated in a water bath at 80 °C for 30 min. The extract was recovered by centrifuging at 10,000 rpm for 10 min, and then the supernatant was used to detect protein and polysaccharide by using the Bradford [6] and phenol-sulfate methods [11], respectively.

CH_4 oxidation rate measurement

CH_4 oxidation rate was measured using the method described by Wang et al. [42]. In brief, after CH_4 injection, four gas samples were taken periodically from the headspace of the serum bottles. Then, the CH_4 oxidation rates were calculated by the regression of the amount of CH_4 loss (*i.e.* the amount of CH_4 loss in a non-microbial control was subtracted from that in the treatment) against time. The CH_4 concentrations were measured using a gas chromatograph equipped with a thermal conductivity detector (TCD). The gas chromatography conditions for the TCD were as follows: two 2 m stainless steel columns filled with GDX-103 (80/100 mesh) and 5A molecular sieves, respectively; the temperatures of the oven, injector and detector were 90 °C, 100 °C and 100 °C, respectively. Hydrogen with a 99.999% purity was used as the carrier gas and it was introduced at a flow rate of 20 mL min^{-1}.

DNA extraction, Q-PCR and T-RFLP analysis

DNA was extracted from 0.5 g soil samples with the E.Z.N.A.™ Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, USA). The experimental triplicates of each soil sample were used for DNA extraction, respectively. DNA was quantified using the Nanodrop ND-1000 spectrophotometer, and it was then mixed in equal amounts to obtain the DNA for each treatment and sampling time point sample for Q-PCR and T-RFLP analysis. The *pmo*A gene was amplified using