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Coupled methane and nitrous oxide biotransformation in freshwater wetland sediment microcosms

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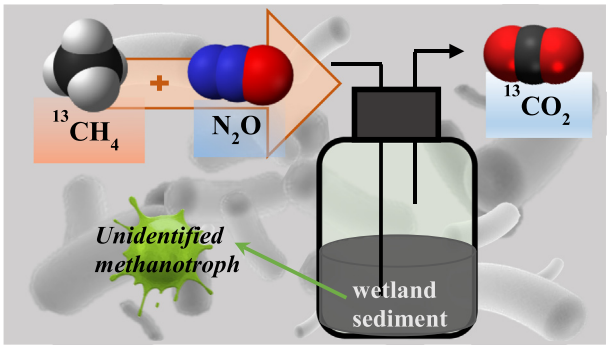
HIGHLIGHTS

- Coupled CH_4 and N_2O biotransformation in a freshwater wetland sediment
- Process carried out by unidentified methanotroph(s) via intra-oxygen production
- Two mechanisms: inhibition of methanogenesis and N_2O-dependent AOM proposed
- The N_2O-dependent AOM rate was 3.41 ± 0.13 nmol CO_2 g^{-1} dry sediment·day^{-1}.
- Activity and abundance of methanotrophic bacteria increased by N_2O addition

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic oxidation of methane (AOM) coupled to denitrification is becoming the focus of scientific inquiry due to its potential contribution to global carbon and nitrogen cycles. AOM has been previously reported to proceed with nitrate (NO_3^-) or nitrite (NO_2^-). However, little research has been conducted on the simultaneous use of methane (CH_4) and nitrous oxide (N_2O). Here, coupled CH_4 and N_2O biotransformation in a freshwater wetland sediment was obtained in a 7-day anaerobic sediment incubation assay. The significant CO_2 accumulation and decrease of CH_4 emission in sediment microcosms was attributed to two mechanisms: inhibition of methanogenesis and N_2O-dependent AOM. To further confirm the coupled CH_4 and N_2O transformation, a ^{13}C-labelled stable isotope tracer assay after anaerobic incubation was conducted with N_2O and/or CH_4 amendments. The N_2O-dependent AOM rate was 3.41 ± 0.13 nmol CO_2 g^{-1} dry sediment·day^{-1}. According to metagenomic analysis, addition of N_2O stimulated AOM by increasing the activity and abundance of methanotrophic bacteria and by increasing enzymatic activities in the electron transport chain. Based on these results, we propose coupled CH_4 and N_2O biotransformation in the sediment microcosms for the first time, carried out by unidentified methanotroph(s) via intra oxygen produced in the presence of N_2O. Such a process has the potential to reduce the emission of two highly potent greenhouse gases and makes a significant contribution to the link of global carbon and nitrogen cycles in anoxic environments.

1. Introduction

Sediments of freshwater wetlands and peatlands are the largest natural source of methane (CH_4) and nitrous oxide (N_2O) emissions to the

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atmosphere (Bastviken et al., 2011; Schlesinger, 2013). As two main greenhouse gases, CH_4 is more potent than CO_2 over century time scales, and N_2O is an activator of ozone destruction in the stratosphere. The current CH_4 and N_2O budgets show an excess of sources over sinks (Bastviken et al., 2011; Schlesinger, 2013). CH_4 is formed by methanogenic archaea in anaerobic environments (Conrad, 2009). Some CH_4 is oxidized by aerobic methanotrophic bacteria when it reaches the oxic biosphere, but most of it is lost from the sediments due to ebullition, mixing events or anaerobic oxidation (Delsontro et al., 2010). In anoxic environments, evidence suggests that anaerobic oxidation of methane (AOM) is coupled to denitrification in freshwater wetlands and peatlands (Ettwig et al., 2010; Haroon et al., 2013; Raghoebarsing et al., 2006).

Denitrification is the reduction of NO_3^- and/or NO_2^- to NO, N_2O and N_2 by many bacteria and archaea by using organic and inorganic compounds as electron donors (Philippot, 2002). Under anoxic conditions in organic carbon rich soils, NO_2^- can be reduced to N_2O and N_2 via nitrifier denitrification (Wrage et al., 2001). Recently, CH_4 has been shown to be a favorable electron donor for both NO_3^- and NO_2^- reduction by Islas-Lima et al. (2004), who firstly confirmed the process of AOM coupled to denitrification. Raghoebarsing et al. (2006) enriched a microbial consortium from anoxic sediments and reported that NO_2^- was preferred to NO_3^- as the substrate for denitrification. This microbial consortium consisted of the bacterium Candidatus "*Methylomirabilis oxyfera*", and a novel anaerobic methanotrophic (ANME) lineage, ANME-2d (Haroon et al., 2013). Hu et al. (2014) pointed out that NO_2^--dependent AOM was a previously overlooked microbial CH_4 sink in wetlands. Ettwig et al. (2010) have proposed a pathway of CH_4 oxidation with NO_2^- that bypassed the denitrification intermediate N_2O, in which two molecules of NO were converted to O_2 and N_2. Recently, Khan et al. (2014) reported that synthesis gas ($CO + H_2$) could be formed from CH_4 using N_2O as oxidant catalyzed by Co-ZSM-5. Besides, Parfenov et al. (2014) have reported CH_4 oxidation by N_2O at 200 °C and lower temperatures on FeZSM-5 surface. It is noteworthy that Gupta et al. (2013) used $^{13}CH_4$ tracers to study AOM in peatlands in the United States and concluded that undisclosed electron acceptor (s) must be involved in this process. Whether N_2O could be involved in CH_4 oxidation under anoxic conditions is still unclear.

N_2O, mainly the result of denitrification, may be involved in AOM (Mander et al., 2014). Based on Gibbs free energy calculations, the process of CH_4 oxidation coupled to N_2O reduction (Eq. (1)), is more thermodynamically favorable than coupled with NO_3^- or NO_2^- reduction (Eqs. (2) and (3)). Oxidation of CH_4 coupled with N_2O reduction is thermodynamically feasible, but no direct evidence has been found to date. Therefore, we propose coupled CH4 and N2O biotransformation in the sediments for the first time.

$$CH_4 + 4N_2O \rightarrow CO_2 + 4N_2 + 2H_2O \quad \Delta G^{o'} = -1232.4\ kJ/mol\ CH_4 \tag{1}$$

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O \quad \Delta G^{o'} = -765 kJ/mol\ CH_4 \tag{2}$$

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad \Delta G^{o'} = -928 kJ/mol\ CH_4 \tag{3}$$

The primary purpose of the present study was to assess whether N_2O affects the emission of CH_4 in wetlands, which has been previously overlooked. For this purpose, sediment samples were collected from typical freshwater wetlands to study the synchronous decrease of CH_4 and N_2O. ^{13}C-labelled isotope tracer assays were conducted to confirm that AOM involved N_2O reduction. Real-time PCR (qPCR) and metagenomic sequencing were utilized to explore the mechanism for the N_2O-dependent AOM process.

2. Materials and methods

2.1. Sediment samples

The Xiaomei River surface flow constructed wetland, which has been used for further purification of the effluent from a wastewater treatment plant, was selected for the present study. Xiaomei River, having a history of >560 years, is located in Shandong, China (Liu et al., 2015). At the sampling location, the water depth was 20 cm and the sediment with a depth of about 10 cm was over gravel. Three sediment cores were collected in March 2016 at the water/sediment interface to represent low dissolved oxygen (DO) conditions (DO < 0.8 mg/L), using a Luoyang shovel (Ye et al., 2012) and stored in self-sealing bags, flushed with Ar gas to ensure anoxic conditions. The sediment samples were preincubated at room temperature (22 ± 2 °C) for at least 24 h without any substrate addition to exhaust any labile nutrients (Zhang et al., 2014).

2.2. Analytical methods for sediment characterization

The collected sediment samples were analyzed for a number of parameters listed in Table S1 (Supplementary Material). Organic carbon and pH were measured using a TOC analyzer (Shimadzu, Japan) and a pH meter (pHS-3C), respectively. Nitrate, nitrite, phosphate and COD concentrations were measured using standard methods (Rice et al., 2012). Fe and Mn concentrations were analyzed using ICP-AES (IRIS intrepid II XSP, Thermo Electron, USA).

2.3. Preliminary sediment anaerobic incubation

The preliminary batch incubation was carried out to test the effect of N_2O on anaerobic oxidation of methane. All samples were handled in an anaerobic work station under an Ar gas (99.99% purity) atmosphere. A set of 54, 100 mL serum bottles containing 5 g of sediment (wet weight) mixed with 20 mL distilled water were sealed with butyl rubber septa and shaken at 120 rpm for 7 days at 30 ± 2 °C. Before incubation, the headspace (nearly 80 mL) was flushed with Ar gas (99.99% purity) and left at atmospheric pressure. Three groups were set up as follows: (i) live sediment (labelled as N_2O-negative), (ii) live sediment amended with N_2O (labelled as N_2O-positive), and (iii) autoclaved sediment. Autoclaving was performed three times at 120 °C for 40 min (Navarro et al., 2015). For each bottle of N_2O-positive group and autoclaved sediment group, 10 mL of headspace gas was withdrawn and replaced with an equal volume of N_2O/Ar mixture (1% N_2O in 99% Ar). For each group, triplicate bottles were sampled and sacrificed on day 0, 1, 2, 3, 5 and 7 for gas composition analysis. Headspace gas sample (10 mL) from each bottle was withdrawn and the concentration of CH_4, N_2O and CO_2 was measured using gas chromatography as previously described (Hu and Ma, 2016).

2.4. Isotope tracer assay

Three reactors, amended with CH_4 + Ar, CH_4 + N_2O, and N_2O + Ar, labelled as XMR-C, XMR-CN and XMR-N, respectively, were set up to carry out a ^{13}C-labelled isotope tracer assay. For each reactor, a sediment sample of 300 g (wet weight) was transferred to an Ar-flushed, 2.5 L wide-mouth bottle together with 0.5 L Ar-purged deionized water. Before the transfer, each reactor's headspace was flushed with pure Ar (99.999%) to maintain anoxic conditions. The reactor's contents were mixed by magnetic stirring at 150 rpm. Before the isotope tracer assay, the sediments were preincubated with N_2O and/or CH_4 amendment as shown in Fig. S1. Based on the expected consumption of CH_4 and N_2O determined in preliminary tests, every 24 h, 20 mL CH_4 (1% CH_4 in 99% Ar) and/or 40 mL N_2O (1% N_2O in 99% Ar) was injected into the respective reactors' headspace. A gas bag was connected to the reactor headspace to keep pressure as 1 atm. Incubation took place at 30 ± 1 °C for 30 days based on previously reported research