More evidence that anaerobic oxidation of methane is prevalent in soils: Is it time to upgrade our biogeochemical models?

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ABSTRACT

Estimating future fluxes of CH_4 between land and atmosphere requires well-conceived process-based biogeochemical models. Current models do not represent the anaerobic oxidation of methane (AOM) in land surface soils, in spite of increasing evidence that this process is widespread. Our objective was to determine whether AOM, or potential AOM, commonly occurs in 20 hydromorphic soils spanning a wide range of chemical properties. Bulk soil samples were collected under shallow water near the shoreline of 15 recently drained fish ponds in southern Bohemia (Czech Republic), as well as from below the water table at 3 peatland locations in northeast Scotland and 2 acid sulfate soils on the southern coast of Finland. Each soil slurry was incubated under both oxic and anoxic conditions, with or without the addition of alternative electron acceptors (SO_4^{2-} and NO_3^-) or $H_2PO_4^-$. Here, "oxic" and "anoxic" conditions refer to anoxic soil respectively incubated in a headspace containing air or argon. Using the isotope dilution method, we determined the gross production and oxidation rates of CH_4 after 2 days incubation under oxic headspace conditions, and after 2, 21 and 60 days incubation under anoxic conditions. Large differences in net CH_4 fluxes were observed between soil types and between incubation conditions. AOM was detected in each of the 20 bulk soil samples, which spanned >6 pH units and 2 orders of magnitude in organic C content. Significant positive relationships were found between AOM and gross CH_4 production rates under anoxic conditions, resulting in AOM rates that were sometimes higher than CH_4 oxidation rates under oxic headspace conditions. There was no relationship between net and gross CH_4 production rates, such that 2 soil types could display similar low net rates, yet conceal very large differences in gross rates. The effects of alternative electron acceptors on AOM were idiosyncratic and resulted in no net trend. We did find, however, a negative effect of SO_4^{2-} and $H_2PO_4^-$ on gross CH_4 production rates under anoxic and oxic conditions respectively. Under oxic headspace conditions, CH_4 oxidation was related to soil organic C content. Taken collectively, our results suggest that AOM, or potential AOM, is prevalent over a wide range of soil types, that AOM may contribute substantially to CH_4 oxidation in soils, and that AOM in soils should be integrated to current process-based CH_4 cycling models.

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1. Introduction

Methane (CH_4) is an important greenhouse gas whose infrared absorption capacity over a centennial time-scale is 28 times higher than that of CO_2 (Myhre et al., 2013). Atmospheric CH_4 concentrations have been steadily increasing over the past 200 years, and are projected to keep on increasing under various climate change scenarios (Cubasch et al., 2013). In order to estimate future fluxes of CH_4 between land and atmosphere, a number of process-based biogeochemical models have been developed, such as DNDC (Li et al., 1996), Wetland-DNDC (Zhang et al., 2002), TEM (Zhuang et al., 2004), DAYCENT (Del Grosso et al., 2006) and DLEM (Tian et al., 2011). These models simulate critical biogeochemical processes that control the simultaneous microbial production and oxidation of CH_4 in terrestrial ecosystems. The algorithms used in these models consider many factors such as water table depth, substrate quality and quantity, and/or soil pH. A common premise in nearly all of these models is that CH_4 oxidation is a process carried out by aerobic methanotrophic bacteria above a threshold oxygen concentration. To our knowledge, only one process-based model, TEM (Zhuang et al., 2004), provides an optional algorithm to represent the anaerobic oxidation of methane (AOM).

AOM is a process that was first reported to occur in deep anoxic marine sediments (Reeburgh, 1976). In this environment, CH_4 is

oxidized with sulfate (SO_4^{2-}) as the terminal electron acceptor. It is mediated by a syntrophic consortium formed by SO_4^{2-} reducing bacteria and anaerobic CH_4 oxidizing archaea (Hoehler et al., 1994), or by the latter alone (Milucka et al., 2012). Since this landmark discovery, AOM was found to occur in other environments including freshwater lake sediments and water columns (Eller et al., 2005; Raghoebarsing et al., 2006), mud volcanoes (Alain et al., 2006), landfill leachate (Grossman et al., 2002), deep buried Holocene sediments (Takeuchi et al., 2011) and hydrocarbon contaminated aquifers (Amos et al., 2012). All of these situations point to AOM as being primarily occurring in highly reducing conditions. Thus, observations of AOM in surface soils with fluctuating redox conditions are relatively scarce. More recently there have been, however, a few reports of AOM occurring in surface peatland soils (Smemo and Yavitt, 2007; Zhu et al., 2012), pasture soil (Bannert et al., 2012), a cultivated drained podzol (Pozdnyakov et al., 2011), as well as in boreal and tropical forest soils (Blazewicz et al., 2012). These findings raise the question of whether AOM is much more prevalent in surface soils than was originally thought.

Ongoing research on the microbiology and biochemistry of AOM has uncovered several alternative pathways by which CH_4 may be oxidized in the absence of O_2. Besides SO_4^{2-}, the coupled oxidation of CH_4 may be mediated by other electron acceptors such as manganese (Mn(IV)) or iron (Fe(III)) (Beal et al., 2009), NO_3^- (Raghoebarsing et al., 2006), or by single denitrifying populations that convert nitrite (NO_2^-) to nitric oxide (NO) in order to generate O_2 that is then used internally to oxidize CH_4 (Ettwig et al., 2008). Thus, the experimental addition of various electron acceptors such as SO_4^{2-} or NO_3^- might help reveal potential AOM in various land surface environments.

Compared to the oxidation of CH_4 under oxic conditions, AOM is generally assumed to be a slow process. For one, biochemical rate constants generally decline with a decrease in redox potential. Furthermore, it is reported that only 1% of anaerobically consumed methane is directly channeled into biosynthesis, whereas 99% is oxidized to CO_2 (Knittel and Boetius, 2009). Thus, Raghoebarsing et al. (2006) estimated that the growth rate of microorganisms involved in AOM could be extremely low, with a doubling time on the order of several weeks. In order to gauge the importance of AOM in surface soils, it is therefore necessary to compare AOM with CH_4 oxidation rates of saturated soils under oxic headspace conditions.

Here, we report on a study that was designed to test the prevalence of AOM in 20 independent hydromorphic surface soils sampled in 3 distinct environments in Europe. In order to increase the odds of finding AOM, the experiment also tested for the effects of NO_3^- and SO_4^{2-} amendments, which we hypothesized could stimulate the AOM process. In order to compare the relative efficiency of AOM in these soils, we performed a similar experiment under oxic conditions. Given that SO_4^{2-} has never been reported to stimulate CH_4 oxidation under oxic conditions, but that phosphate has (Jugnia et al., 2012), we substituted SO_4^{2-} for ortho-phosphate ($H_2PO_4^-$) under oxic conditions. The net fluxes of CH_4 and CO_2 were assessed by gas chromatography, whereas the gross production and oxidation rates (i.e. gross transformation rates) of CH_4 were assessed by isotope dilution. Results suggest that AOM in land surface may be prevalent and driven by CH_4 production rates, whereas the effects of NO_3^- or SO_4^{2-} amendments remain unclear.

2. Materials and methods

2.1. Soil sampling and analyses

In spring 2012, we collected 20 hydromorphic surface soil samples from 3 distinct regions in Europe, namely from 15 commercial fish ponds in southern Bohemia (Czech Republic), from 3 locations within an ombrotrophic peatland bog in north-east Scotland, and from 2 coastal acid-sulfate soils in southern Finland. The fish ponds are located within a 30 km radius around the city of České Budějovice, and are part of a 500-year old network of commercial fish ponds that are occasionally fertilized with manure, or drained to harvest fish. The peatland bog is named "The Red Moss of Netherley", and constitutes the largest "raised bog" site in the County of Kincardine (Scotland); an extensive description of its hydrological, chemical and vegetation features is given by Hulme (2006). The acid sulfate soils have recently emerged from the Baltic Sea as a consequence of post-glacial isostatic rebound, and are categorized by relatively high concentrations of carbon (110 Mg ha^{-1}) and sulfur (1.5—2.0 ppm) in the subsoil (1.3—1.8 m) horizons (Šimek et al., 2011).

The sampling criteria were that soil material (*i*) had to be collected from an anoxic soil layer, and (*ii*) was nevertheless close to land surface. Thus, the 15 sampled ponds had been submerged by several metres of water for several years, and were considered to have very low dissolved oxygen due to the combination of high carp densities, repeated manure applications and a lack of running water. Each of these 15 ponds had been drained 1—2 days before we sampled their sediments. Once drained, there remains a large area in the middle of each pond where shallow surface water remains. Each of our bulk pond soil slurries (hereafter referred to as "shoreline soils") were thus collected approximately 10—15 cm below the water surface. For each peatland site, 3 bulk samples were collected from the 100—120 cm deep muck layer using a specialized peat corer. Samples were collected near permanent water level meters, which indicated that the water table was no deeper than 30—50 cm. As for the 2 acid sulfate soils, trenches were mechanically dug and bulk samples were collected from the permanently submerged Cg1 and Cg2 horizons, at a depth of 130—180 m. These acid sulfate soils, located near the Gulf of Finland, were recently equipped with drains that maintain water table depth at 85—100 cm. Because Cg1 and Cg2 horizons are submerged, sulfur remains in a reduced state and pH tends towards neutrality.

Each of the 20 bulk soil slurries were hypersaturated at the time of sampling, with a mean gravimetric moisture content of 97% for the 17 mineral soil samples (i.e. organic C < 17%) and 1176%, for the 3 organic peat samples. Each sample was immediately placed in a plastic vacuum bag and excess air was removed by suction prior to sealing the bag. Bags were then placed in coolers under ice and transported to the Institute of Soil Biology (ISB) in České Budějovice (Czech Republic), where they were kept at 4 °C for no more than 2 weeks.

Soils collected at each site were characterized by selected chemical properties. Air dried subsamples were extracted in a 1.0 N KCl solution, and the extracts were analyzed colorimetrically for ammonium (NH_4^+) and nitrate (NO_3^-) concentrations respectively using the Berthelot (NH_4^+) and the Griess-Illosway (NO_3^-) reaction methods (Mulvaney, 1996). Total C was measured by wet oxidation, as described by Nelson and Sommers (1982). Soil pH was measured in deionized water using a soil:liquid ratio of 1:10 for organic peat samples, and 1:3 for mineral soil samples.

2.2. Testing for the occurrence of AOM

In order to test for the occurrence of AOM, concurrent rates of CH_4 production and oxidation were measured in each soil type using a gaseous isotope dilution method (Bradley et al., 2012) under anoxic conditions and with various soil amendments. Ten glass beads were inserted into a 1 L glass bottle that was then sealed with an air-tight butyl rubber stopper. The headspace air in this bottle