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Stabilization and stimulation of atmospheric methane oxidation in soil and soil biofilters by Al_2O_3 amendment

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ABSTRACT

Oxidation of atmospheric methane by soil methanotrophs is a microbial process highly susceptible to physical and chemical disturbances. In this study, atmospheric methane oxidation activity in soil samples from beech (*Fagus sylvatica*) and spruce (*Picea abies*) forest stands decreased significantly after physical disturbance and/or increased exposure to ambient air. This activity loss was oxygen dependent, but independent of the presence of atmospheric methane. However, methanotrophic activity in forest soil was stabilized by amendment with various sorbents including activated charcoal, aminopropylsilane, and gamma aluminum oxide (γ-Al_2O_3). γ-AlO_3 at a concentration >1% (g g^{-1}) was found to stabilize and sometimes stimulate oxidation of atmospheric methane in soil from both beech and spruce forest stands. γ-Al_2O_3 amendment also stimulated atmospheric methane oxidation in advective flow-based soil biofilters, and the filter efficiency was found to increase with time. In both soil samples and soil biofilters, elevated oxidation of atmospheric methane was sustained for >100 days. γ-Al_2O_3 likely immobilized potentially inhibitory soil constituents including inorganic nitrogen and soil organics. The results of the study indicated that: 1) decreases in atmospheric methane oxidation activity in topsoil after soil homogenization and/or increased air exposure was likely related to increased bioavailability of inhibitory substances; 2) indigenous inhibitory compounds are present in topsoil in both beech and spruce forest soil; and 3) oxidation of atmospheric methane in soil can be restored and sometimes stimulated by immobilizing inhibitory compounds using γ-Al_2O_3 as sorbent.

1. Introduction

Methane is a key greenhouse gas accounting for about 18% of the overall global radiative forcing (Forster et al., 2007; Blagodatsky and Smith, 2012). The atmospheric methane concentration has increased for several decades due to an imbalance of 28–37 Tg yr^{-1} between methane emission and consumption (Solomon et al., 2007; Dalal et al., 2008). This imbalance is more or less equal to the annual microbial oxidation of atmospheric methane in soils which accounts for 15–45 Tg yr^{-1}, making atmospheric methane oxidation in soil a very important process in the global methane cycle (Dalal et al., 2008; Conrad, 2009; Curry, 2009; Kolb, 2009).

Soil atmospheric methane oxidation (SAMO) has been observed in a range of terrestrial environments, and is likely mediated by groups of high affinity methanotrophic bacteria (Bender and Conrad, 1992; Holmes et al., 1999; Roslev and Iversen, 1999; Knief and Dunfield, 2005; Kolb et al., 2005; McDonald et al., 2008; Kolb, 2009). These intriguing high affinity methanotrophs have not yet been cultured and characterized in detail, and they appear unique compared to known methanotrophic bacteria from culture collections (Bender and Conrad, 1992; Roslev et al., 1997; Holmes et al., 1999; Knief and Dunfield, 2005; Kolb et al., 2005; McDonald et al., 2008; Conrad, 2009; Kolb, 2009). Unfortunately, these atmospheric methane oxidizers appear to be slow growing, and soil methane oxidation activity is sensitive to physical disturbances both in situ and in the laboratory (Mosier et al., 1991; Kruse and Iversen, 1995; Dobbie and Smith, 1996; Prieme et al., 1997; Roslev et al., 1997; Sitaula et al., 2000; Dalal et al., 2008). For example, significant loss of methane oxidation activity has been observed in laboratory experiments after soil mixing or sieving (Roslev et al., 1997). However, the explanation for the occasional loss of SAMO activity after physical disturbances is not entirely clear, and cannot alone be explained by changes in gas diffusivity or soil water content.

Soil chemical factors such as ammonia, nitrate and nitrite or certain organic compounds have been shown to significantly affect

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SAMO activity (Roslev et al., 1997; Bodelier and Laanbroek, 2004; Crossman et al., 2006; Dalal et al., 2008; Maurer et al., 2008; Menyailo et al., 2010; Mochizuki et al., 2012). Several natural organic compounds present within topsoil have been found to inhibit SAMO including monoterpenes and phenolic acids (Amaral and Knowles, 1997, 1998; Maurer et al., 2008; Kolb, 2009). These organic inhibitory compounds are most likely responsible for SAMO to be a subsurface phenomenon in some soil types (Amaral and Knowles, 1998; Kolb, 2009). Collectively, these results indicate that several chemical factors may have a substantial negative effect on SAMO activity. Despite this knowledge about factors inhibiting microbial oxidation of atmospheric methane in soil, detailed insights including means to alleviate the potential inhibitory effects are lacking.

The purpose of this study was therefore to examine factors that cause loss of SAMO activity in soil, and to determine if atmospheric methane oxidation activity in soil can be stabilized or even stimulated by addition of sorbents to offset the inhibitory effects of soil solutes. Various sorbents were evaluated using soil cores and soil samples from different vegetation types and soil depths, and the most promising sorbent was evaluated further in long term enrichment experiments with soil samples and advective flow-based soil filters exposed to atmospheric methane.

2. Materials and methods

2.1. Field sites

Soil samples were collected in Rold Forest, which is located in the northern part of Jutland, Denmark. Rold Forest has an area of 80 km^2 and was originally vegetated with beech until 1850, where spruce and pine trees were introduced. Two experimental sites were selected for these experiments: one vegetated with European beech (*Fagus sylvatica*) (N 56-50-680, E 009-50-908), planted in 1919, and one vegetated with Norwegian spruce (*Picea abies*) (N 56-50-744, E 009-51-328), planted in 1964. Samples from these sites will be denoted as the Beech and the Spruce soils, respectively. The sand and silt dominated soils were classified as fragiorthods (fragic podzols), and the O, A and E horizons were generally included in sampling of intact soil cores (see below). Selected physical and chemical parameters for the two soils are shown in Table 1. Soil water, mineral and organic content were determined by drying and loss of ignition for 24 h at 105 °C, and 8 h at 550 °C, respectively. Volumetric distribution of soil volumes were calculated based on bulk density, soil component gravimetric distribution and their assumed densities (g cm^{-3}) of: water 1, organic material 1.1, and minerals 2.65. pH and concentrations of NH_4^+, NO_2^-, and NO_3^- were determined after extraction with 1 N KCl.

Table 1
Some chemical and physical properties of the soil at the field sites vegetated by beech (*Fagus sylvatica*) and spruce (*Picea abies*) ($n = 6$).

	Soil depth (cm)			
	0–5	5–10	10–15	15–20
Beech				
Porosity (cm^3 cm^{-3})	0.76	0.56	0.55	0.54
Organic matter (g kg^{-1} dw)	245	50	35	35
pH (KCl)	4.46	4.31	4.46	4.72
NH_4^+ (mg N kg^{-1} dw)	16.43	5.46	4.80	3.49
NO_2^- (mg N kg^{-1} dw)	0.01	0.02	0.01	0.01
NO_3^- (mg N kg^{-1} dw)	4.14	2.53	1.34	0.86
Spruce				
Porosity (cm^3 cm^{-3})	0.92	0.79	0.65	0.57
Organic matter (g kg^{-1} dw)	625	375	170	75
pH (KCl)	3.61	3.55	3.72	4.00
NH_4^+ (mg N kg^{-1} dw)	21.05	9.23	3.55	1.82
NO_2^- (mg N kg^{-1} dw)	0.05	0.03	<0.01	<0.01
NO_3^- (mg N kg^{-1} dw)	3.45	1.85	0.69	0.34

2.2. Methane oxidation in soil

Intact soil cores were collected from the Beech and Spruce sites, with 30 cm PVC cores (Ø10 cm). The soil cores were brought back to the lab, and stored in the dark for a maximum of 7 days at 10 ± 2 °C until initiation of experiments. Atmospheric methane uptake rates for intact soil cores were determined by closing the cores with a lid equipped with butyl rubber seals, and a septum for gas sampling. Headspace gas samples of 0.3 cm^3 were withdrawn with needle and syringe, and the methane concentration determined as described below. All soil cores were temperature equilibrated for 12 h at 20 ± 2 °C before flux measurements.

Atmospheric methane oxidation rates for soil samples were determined for 20 g fresh soil in 58 cm^3 glass vials incubated with ambient air. Soil samples originated from intact cores sectioned into 4 cm layers, and unless stated otherwise passed through a 4 mm mesh (hereafter referred to as "sieved soil samples"). Headspace gas samples of 0.3 cm^3 were withdrawn with needle and syringe, and the methane concentration determined as described below. Oxidation rates were determined immediately after sieving of soil samples, and at different time intervals post sieving (see Section 2.4). Headspace methane concentrations in intact cores and in vials with soil samples were analyzed on a Chrompack 438A gas chromatograph equipped with a flame ionization detector. The injector, oven, and detector temperatures were, 110 °C, 80 °C and 240 °C, respectively. Gases were separated on a 200 × 2 mm 80/100 mesh Hyaesep Q column with N_2 as carrier gas. Methane concentrations were calculated from known standards. The lower level of detection for CH_4 was 0.1 ppmv. The atmospheric methane oxidation flux for intact cores (F) and the atmospheric methane oxidation rates for soil samples (R) were calculated by fitting measured methane concentration to exponential functions based on first order oxidation kinetics.

2.3. Experimental overview

Three main topics were examined in this study (Table 2): 1. "Storage impact" served to clarify how SAMO activity was affected by soil storage and the storage atmosphere, 2. "Sorbent impact" served to test the hypothesis that loss of SAMO activity in some soil samples is related to inhibitory compounds removable through sorption, and 3. "Al_2O_3 impact" was conducted to examine concentration and long term effects of laboratory Al_2O_3 amendment on SAMO activity.

2.4. Impact of soil storage on methane oxidation

The effect of soil storage and storage atmosphere on the potential for oxidation of atmospheric methane was examined for intact soil cores and sieved soil samples. Soil cores were incubated in ambient air at 20 ± 2 °C, and methane fluxes determined at different time intervals (F_t) where t denotes the time since the start of incubation.

Sieved soil samples were incubated at 20 ± 2 °C in glass containers (50 L) continuously flushed with controlled gas mixtures with different composition to examine the effect of ambient oxygen and methane on the potential for oxidation of atmospheric methane. The controlled gas mixtures (Norsk Hydro, Statoil, Norway) included artificial air without methane (800 pptv N_2, 200 pptv O_2 and <0.1 ppmv CH_4) hereafter referred to as "$-CH_4+O_2$", and artificial air without oxygen and methane (1000 pptv N_2, <10 ppmv O_2 and <0.1 ppmv CH_4) hereafter referred to as "$-CH_4-O_2$".