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Endogenous Methanogenesis Stimulates Oxidation of Atmospheric CH_4 in Alpine Tundra Soil

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Abstract

Experiments were done to test the hypothesis that atmospheric CH_4 oxidizers in a well-drained alpine tundra soil are supported by CH_4 production from anaerobic microsites in the soil. Soil was subjected to 22 days of anaerobic conditions with elevated H_2 and CO_2 in order to stimulate methanogenesis. This treatment stimulated subsequent atmospheric CH_4 consumption, probably by increasing soil methanogenesis. After removal from anaerobic conditions, soils emitted CH_4 for up to 6 h, then oxidized atmospheric CH_4 at 111 (±5.7) pmol (g dry weight)$^{-1}$ h^{-1}, which was more than 3 times the rate of control soils. Further supporting our hypothesis, additions of lumazine, a highly specific inhibitor of methanogenesis, prevented the stimulation of atmospheric CH_4 oxidation by the anaerobic treatment. The method used to create anaerobic conditions with elevated H_2 and CO_2 also elevated headspace CH_4 concentrations. However, elevated CH_4 concentrations under aerobic conditions did not stimulate CH_4 oxidation as much as preexposure to H_2 and CO_2 under anaerobic conditions. Anaerobic conditions created by N_2 flushing did not stimulate atmospheric CH_4 oxidation, probably because N_2 flushing inhibited methanogenesis by removing necessary precursors for methane production. We conclude that anaerobic conditions with elevated H_2 and CO_2 stimulate atmospheric CH_4 oxidation in this dry alpine tundra soil by increasing endogenous CH_4 production. This effect was prevented by inhibiting methanogenesis, indicating the importance of endogenous CH_4 production in a CH_4-consuming soil.

Introduction

Microorganisms in well-drained soils consume the greenhouse gas CH_4 from the atmosphere, and are the second largest sink in the global CH_4 budget [21, 22]. These microbes appear to utilize atmospheric CH_4 for biomass production [23] and to be related, but not identical, to type II methanotrophs which have been studied in pure culture [2, 8, 10, 13, 15, 24]. The continuous activity of atmospheric CH_4 oxidizers in soils implies that they survive on atmospheric CH_4 concentrations of 2 ppmv, but they do not appear able to grow on such low CH_4 concentrations [2,25]. It has been suggested that they

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utilize other carbon substrates such as methanol, formate, and acetate in addition to atmospheric CH_4 for their growth [5, 12, 16, 23, 27]. It has also been suggested that atmospheric CH_4 oxidizers may utilize endogenous CH_4 production from anaerobic microsites of otherwise aerobic soils [1, 9, 14, 26, 27, 30].

West and Schmidt [26] found that wetting stimulated atmospheric CH_4 oxidation in a well-drained alpine tundra soil, possibly by stimulating methanogenesis inside anaerobic soil microsites, which, in turn, resulted in greater populations or activity of atmospheric CH_4 oxidizers. Supporting this explanation, atmospheric CH_4 oxidation in this soil was also stimulated by acetate [27], a substrate likely to be utilized by methanogens but not by methanotrophs. Despite this evidence for the importance of endogenous CH_4 production, CH_4 emission has not been observed in the field from this soil [28] or from similar dry alpine soils [17, 29]. To further explore the possibility that endogenous CH_4 production supports the atmospheric CH_4 oxidizers of this soil, we tested the effects of anaerobic conditions with elevated H_2 and CO_2 on subsequent atmospheric CH_4 oxidation. We also used the methanogenic inhibitor lumazine [19] to determine whether these effects were mediated by methanogenesis.

Materials and Methods

This study was conducted on soils from alpine tundra of Niwot Ridge, the Long Term Ecological Research (LTER) Site in the Colorado Front Range. This site, its soils, and its CH_4 fluxes are discussed elsewhere [11, 28]. Most experiments of this study were conducted on soil from a dry, CH_4-oxidizing plant community, dominated by the plant *Kobresia myosuroides* (Table 1). In addition, we conducted some tests on soil from a wet, CH_4-emitting plant community dominated by the plant *Carex scopulorums* (Table 1). The *Kobresia* meadow soil used in this study was taken from pieces of intact tundra (approximately $30 \times 30 \times 20$ cm) that were stored in an environmental chamber simulating spring conditions [26]. These pieces were watered regularly to prevent soils from drying excessively. They were not used for an experiment unless plants were green. The *Carex* meadow soil used in this study was collected in June 1997 and stored at 3°C until May 1998. Before each experiment, *Kobresia* meadow soil was sieved (2 mm) and *Carex* meadow soil was homogenized by hand. Soil moisture was determined by drying to constant weight at 100°C. *Kobresia* meadow soil moistures were 63, 64, and 48% H_2O, for the first, second, and third experiments, respectively. *Carex* meadow soil moistures ranged from 50 to 100%.

Methane oxidation or production was measured with room-temperature incubations of soil subsamples (3 replicates each treatment). Soil (8, 10, 15, or 25 g dry weight) was placed in specimen cups inside 1 L mason jars fitted with Teflon-coated silicone septa. Before each measurement of net CH_4 flux, mason jars were opened and allowed to equilibrate with atmospheric CH_4 concentrations (usually between 1.8 and 2.0 ppm). Gas samples were taken at regular intervals with 10 cc syringes, until steady linear increase or decrease of CH_4 concentrations could be confirmed. Preliminary experiments showed that CH_4 consumption by these soils did not differ significantly from linear uptake. Syringes were disassembled to equilibrate with atmospheric air for a minimum of 14 h before each new use. Methane samples were analyzed on a Hewlett Packard 5890A gas chromatograph using a flame ionization detector (FID) at 150°C. Variability of repeated injections of a 1751.8 ppb CH_4 standard calibrated by the National Center for Atmospheric Research (NCAR) was ±10 ppb or less. Rates of CH_4 oxidation or production were calculated by linear regression of the changes in CH_4 concentrations over time, correcting for temperature and pressure. Methane oxidation is presented in figures as negative CH_4 flux.

We conducted three experiments that tested the effect of anaerobic conditions with elevated H_2 and CO_2 on atmospheric CH_4 oxidation in *Kobresia* meadow soil. For the $-O_2+H_2+CO_2$ treatment, a palladium-catalyzed reaction was used to create a strictly anaerobic environment [7]. Each soil subsample was

Table 1. Soils and treatments used in the experiments and tests of this study

Experiment	Soil type	Treatments	Figure
1	*Kobresia*	$-O_2+H_2+CO_2$ $+O_2$ control $+CH_4+O_2$ N_2-flushed	1 and 2
2	*Kobresia*	$-O_2+H_2+CO_2$ $+O_2$ control	3
3	*Kobresia*	$-O_2+H_2+CO_2$ plus lumazine $-O_2+H_2+CO_2$ plus water $+O_2$ plus water control	4
Test of	**Soil type**	**Treatments**	**Table**
CH_4 oxidation	*Kobresia*	$+O_2$ control N_2-flushed	2
CH_4 production	*Carex*	$+O_2$ control N_2-flushed	3
Effects of lumazine on CH_4 flux	*Kobresia* and *Carex*	+lumazine +water control	4
Effects of lumazine on CO_2 production	*Kobresia* and *Carex*	+lumaxine +water control	4

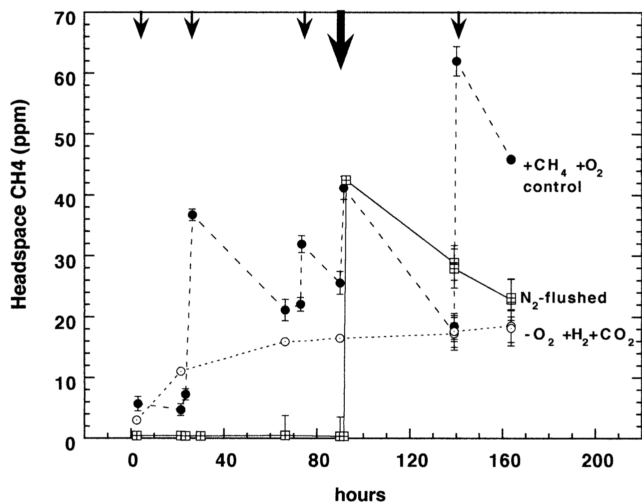

Fig. 1. Headspace CH_4 concentrations during the $-O_2$+H_2+CO_2, +CH_4+O_2 control, and N_2-flushing treatments (shaded area of Fig. 2). Methane accumulated in the headspace of the $-O_2$+H_2+CO_2 treatment. Methane was added to the headspace of the +CH_4+O_2 control treatment in order to keep headspace concentrations at least as high as those in the $-O_2$+H_2+CO_2 treatment. Methane had to be added repeatedly (small arrows) because +CH_4+O_2 soils consumed CH_4 at approximately 1 nmol CH_4 g^{-1} h^{-1} (Table 1). Methane was added to the headspace of N_2-flushed (treatment near the end of the experiment (large arrow) in order to determine the quantity of CH_4 consumed under anaerobic headspace (Table 1).

placed in a mason jar with a 30 ml glass scintillation vial containing 0.4 g $NaBH_4$ and 1.5 g $NaHCO_3$. As water was added to each scintillation vial, jars were promptly closed. This procedure removed O_2 from the jar headspace and elevated CH_4, CO_2, and H_2 concentrations to a minimum of 20 ppm, 10%, and 20%, respectively. Experimental soil samples remained under these anaerobic conditions for 6, 22, and 6 days for the first, second, and third experiment, respectively. For the +O_2 control treatment, the palladium catalyst procedure was performed, but 12 h later the headspace was replaced with lab air.

In the first experiment, CH_4 was added to a +CH_4+O_2 control treatment to determine the effect of the CH_4 which accumulated in the headspace of the $-O_2$+H_2+CO_2 treatment. Methane had to be added repeatedly, because +CH_4+O_2 control soils consumed approximately 1 nmol CH_4 (g d.w.)$^{-1}$ h^{-1}. To determine the importance of increased H_2 and CO_2, jars containing soil subsamples were made anaerobic by evacuating and flushing the jars three times with industrial-grade N_2 gas instead of using the palladium catalyst method. Anaerobic conditions in $-O_2$+H_2+CO_2 soils and N_2-flushed treatments were confirmed with methylene blue indicators that turned white to indicate less than 0.5% O_2. To determine the rate of CH_4 consumption occurring in N_2-flushed soils, CH_4 was added to the headspace, and headspace samples were taken at regular intervals. We also measured CH_4 consumption in +CH_4+O_2 control soils by tracking depletion of CH_4 over time. In the second experiment, +CH_4+O_2 and N_2-flushed controls were not conducted.

In the third experiment, we used the methanogenic inhibitor lumazine to confirm whether the effects of anaerobic treatments in *Kobresia* soils resulted from increased methanogenesis. Lumazine (0.1 g) (Sigma-Aldrich Corporation) was dissolved in 80 mL distilled H_2O. Before undergoing the anaerobic treatment, this solution was added to one set of $-O_2$+H_2+CO_2 soils so that the final concentration of lumazine in the soil solution was 1 mM. Lumazine was added to $-O_2$+H_2+CO_2+LZ soils and an equal amount of deionized water was added to $-O_2$+H_2+CO_2 and +O_2 control soils. Then $-O_2$+H_2+CO_2+LZ and $-O_2$+H_2+CO_2 soils were subjected to a 6-day $-O_2$+H_2+CO_2 treatment. Subsequent effects on atmospheric CH_4 oxidation were measured as described above.

We tested the effect of N_2-flushing on CH_4 production in *Carex* meadow soils, in which CH_4 production is easily measurable [28]. Soils (3 replicates of 10 g d.w.) were amended with 10 mM sodium acetate (final concentration in soil solution) to establish high rates of CH_4 production. Then jar headspace was either equilibrated with room air to make the headspace aerobic, or flushed with nitrogen gas to make the headspace anaerobic. Headspace samples were taken regularly to assess CH_4 production as described above.

We tested the effect of the methanogenic inhibitor lumazine [19] on CO_2 production and CH_4 metabolism in both *Carex* and *Kobresia* meadow soils. Lumazine solution (0.1 g dissolved in 80 mL distilled H_2O) was added to soils so that the final concentration of lumazine solution was 1 mM. Control soils were wetted with an equivalent amount of distilled water. The effect of lumazine on soil CO_2 production in both *Carex* and *Kobresia* meadow soils was measured using an infrared gas analyzer (LI-COR 6400). For the CH_4 measurements, *Kobresia* soils were wetted to stimulate atmospheric CH_4 oxidation. As CH_4 oxidation returned to unstimulated rates, lumazine was added and CH_4 oxidation rates were measured. *Carex* meadow soils were amended with 30 μg g^{-1} acetate in order to increase methanogenesis, and soil CH_4 production was measured. Four days later, lumazine was added, and CH_4 production was measured again. To control for inter-subsample variation, these results are presented as the per cent of CH_4 flux that remained after lumazine additions.

Results

In order to determine whether we could increase atmospheric CH_4 oxidation in a tundra soil by increasing the anaerobic microsites suitable for methanogenesis, we subjected soils to anaerobic treatments. In the first experiment, subsamples of dry alpine tundra soil (Kobresia soil) were subjected to differential treatments for 6 days. During this time, CH_4 accumulated in the headspace of $-O_2$+H_2+CO_2 jars (Fig. 1). To control for the effect of this

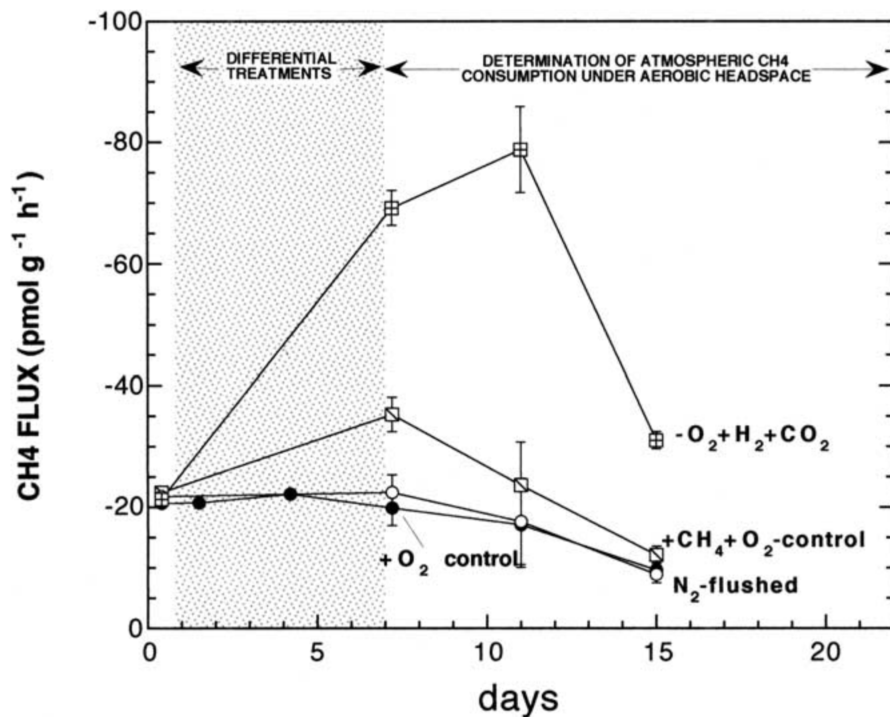

Fig. 2. Atmospheric CH_4 oxidation before and after the differential treatments shown in Fig. 1. Soil subsamples were subject to differential treatments for 6 days (shaded area). After the treatments, atmospheric CH_4 oxidation in $-O_2$+H_2+CO_2 soils increased to 4 times that of +O_2 controls soils. In +CH_4+O_2 control soils, atmospheric CH_4 oxidation increased to nearly twice that of +O_2 control soils. Atmospheric CH_4 oxidation in N_2-flushed controls was not stimulated. Negative CH_4 flux is CH_4 consumption.

extra CH_4, we added CH_4 to the headspace of +CH_4+O_2 control soils. Methane had to be added repeatedly (Fig. 1, small arrows) because +CH_4+O_2 soils consumed approximately 1 nmol CH_4 g^{-1} h^{-1}. N_2-flushed soils did not emit any CH_4, despite being under anaerobic headspace. To test whether these soils could be consuming CH_4, we injected CH_4 into the N_2-flushed headspace (Fig. 1, large arrow) and measured the rate of consumption. Soils under N_2-flushed headspace consumed CH_4 at about half the rate of soils under +CH_4+O_2 headspace (Table 2). Using *Carex* meadow soil, we tested the effect of N_2-flushing on CH_4 production, and found that N_2-flushing inhibited CH_4 production (Table 3).

The 6-day treatments had significant effects on atmospheric CH_4 consumption (Fig. 2). Before treatments, all soils oxidized atmospheric CH_4 at approximately 20 pmol g^{-1} h^{-1}. Afterwards, $-O_2$+H_2+CO_2 treatments had increased atmospheric CH_4 oxidation to nearly 70 pmol (g d.w.)$^{-1}$ h^{-1}, and +CH_4+O_2 treatments had increased atmospheric CH_4 oxidation to 35.3 (±1.7) pmol (g d.w.)$^{-1}$ h^{-1}. Methane oxidation rates in +CH_4+O_2 control soils dropped steadily over the next 8 days, whereas atmospheric CH_4 oxidation in $-O_2$+H_2+CO_2 soils increased to 78.8 (±7.1) pmol (g d.w.)$^{-1}$ h^{-1} before declining. The +O_2 control treatment and N_2-flushing treatment did not change atmospheric CH_4 oxidation significantly.

In a second experiment (Fig. 3), fresh subsamples of *Kobresia* soil were subjected to a longer anaerobic treatment (22 days). For 6 h after removal from the anaerobic environment, $-O_2$+H_2+CO_2 soils emitted CH_4, averaging 11.9 (±7.2) pmol (g d.w.)$^{-1}$ h^{-1}. Subsequently, soils began to consume CH_4, oxidizing 111 (±5.7) pmol CH_4 (g d.w.)$^{-1}$ h^{-1} 5 days later. In contrast, CH_4 oxidation rates in +O_2 control soils decreased from 33.0 (±0.6) to 25 (±1.3) pmol (g d.w.)$^{-1}$ h^{-1} over the course of the experiment.

In the third experiment, we used the methanogenic inhibitor lumazine to determine whether $-O_2$+H_2+CO_2

Table 2. CH_4 oxidation rates in soils under aerobic or N_2-flushed headspace (average of 3 replicates ± standard error)

Headspace	CH_4 oxidation (pmol (g d.w.)$^{-1}$ h^{-1})	Initial [CH_4] (ppm)
Aerobic	1026.0 (±6.1)	41.2 (±1.9)
N_2-flushed	496.7 (±53.3)	37.9 (±1.5)

Table 3. CH_4 production in *Carex* meadow soils under aerobic or N_2-flushed headspace (average of 3 replicates ± standard error)

Day of incubation	CH_4 production [nmol (g d.w.)$^{-1}$$h^{-1}$]	
	Aerobic	N_2-flushed
1	1.8 (±0.4)	1.0 (±0.2)
2	2.1 (±0.6)	1.5 (±0.2)
3	3.0 (±0.5)	1.7 (±0.2)

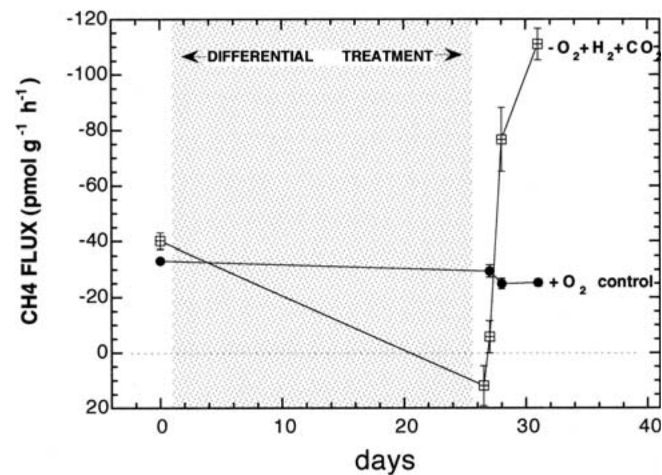

Fig. 3. Oxidation of atmospheric levels of CH_4 before and after a 22-day $-O_2$+H_2+CO_2 treatment. Upon removal from anaerobic conditions, soils briefly emitted CH_4. Then, atmospheric CH_4 oxidation increased to more than 3 times the rate of +O_2 control soils. Negative CH_4 flux is CH_4 consumption.

treatments stimulated atmospheric CH_4 oxidation by increasing acetogenesis or methanogenesis. Lumazine was added to $-O_2$+H_2+CO_2+LZ soils and deionized water was added to $-O_2$+H_2+CO_2 and +O_2 control soils (Fig. 4, arrow). Then $-O_2$+H_2+CO_2+LZ and $-O_2$+H_2+CO_2 soils were subjected to a 6-day $-O_2$+H_2+CO_2 treatment (Fig. 4, shaded area). Wetting alone stimulated atmospheric CH_4 oxidation in +O_2 control soils, but this stimulation was declining after 6 days. Atmospheric CH_4 oxidation in $-O_2$+H_2+CO_2 soils was stimulated approximately 1 week after $-O_2$+H_2+CO_2 treatment, but this stimulation was prevented in soils that had received lumazine.

We conducted tests to ensure that lumazine inhibits methanogenesis even in a complex soil environment, and that lumazine does not inhibit other soil microbes such as atmospheric CH_4 oxidizers (Table 4). We tested the effects of lumazine on methanogens and methanotrophs in alpine soils by adding lumazine to alpine soils that were producing or consuming CH_4. Lumazine inhibited CH_4 production by *Carex* meadow soil ($p < 0.05$), but not CH_4 consumption in *Kobresia* meadow soil (Table 4). To test the effects of lumazine on general microbial activity in these soils, we measured the effects of lumazine on CO_2 production. Lumazine did not inhibit CO_2 production of either *Carex* or *Kobresia* meadow soil (Table 4).

Table 4. The effect of lumazine on CO_2 and CH_4 metabolism of *Carex* and *Kobresia* meadow soils.[a]

	CO_2 production (ppm (15 g d.w.)$^{-1}$min^{-1})	
Soil type	With 1 mM lumazine	Control
Carex	4.1 (±0.6)	4.9 (±0.4)
Kobresia	1.8 (±0.1)	1.8 (±0.1)
	CH_4 production (% previous rate)	
	With 1 mM lumazine	Control
Carex	38.2 (±4.0)	86.6 (±19.5)
	CH_4 consumption (% previous rate)	
	With 1 mM lumazine	Control
Kobresia	32.5 (±3.2)	33.0 (±0.7)

[a] Fluxes of CO_2 were measured with a LICOR 6400 after soils were amended with 1 mM lumazine. Methane fluxes were measured before and after 1 mM lumazine was added. Rates are the average of 3 replicates (± standard error).

Discussion

The results presented here show that atmospheric CH_4 oxidizers of *Kobresia* meadow soil rely on endogenous CH_4 production for growth and maintenance. Activity of atmospheric CH_4 oxidizers was stimulated by exposing this soil to conditions that were designed to stimulate growth of

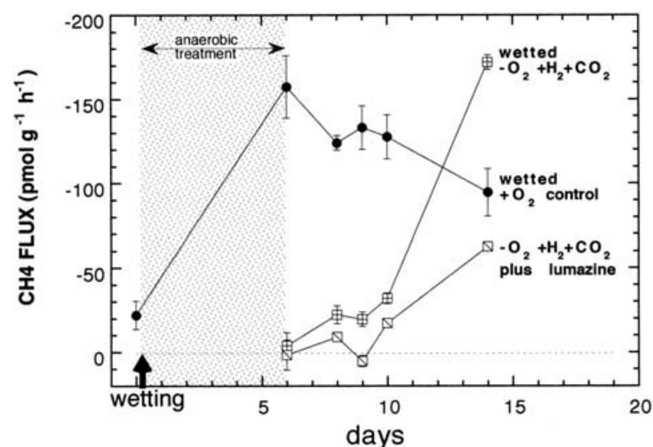

Fig. 4. The methanogenic inhibitor lumazine prevented stimulation of atmospheric CH_4 oxidation by $-O_2$+H_2+CO_2 treatment. Lumazine or deionized water was added to soils (arrow) before they were subjected to $-O_2$+H_2+CO_2 treatment (shaded area). Wetting stimulated atmospheric CH_4 oxidation in +O_2 control soils, but this stimulation began to decline after 6 days. Eight days after exposure to aerobic headspace, atmospheric CH_4 oxidation rates in $-O_2$+H_2+CO_2 soils became stimulated relative to +O_2 control soils, but this stimulation was prevented in $-O_2$+H_2+CO_2 soils treated with lumazine.

methanogens (Figs. 2, 3, and 4). We reduced oxygen concentrations in the $-O_2+H_2+CO_2$ and in the N_2-flushed treatments to less than 0.5%. The reduction in oxygen availability was expected to increase occurrence of the anaerobic microsites where we have hypothesized that methanogenesis occurs [26, 27]. Additions of H_2 and CO_2 were expected to supply carbon and energy to methanogens. Without stimulation by either $-O_2+H_2+CO_2$ treatments, wetting [26], or additions of CH_4 (Fig. 2), atmospheric CH_4 oxidation gradually declined (Figs. 2 and 3).

The most likely mechanism by which $-O_2+H_2+CO_2$ treatments stimulated atmospheric CH_4 oxidation was by increasing the supply of CH_4 to soil methanotrophs. Apparent CH_4 production during the $-O_2+H_2+CO_2$ treatments (Fig. 1), plus the transient CH_4 emission upon removal from the 22-day $-O_2+H_2+CO_2$ treatment (Fig. 3), suggested that CH_4 was produced under $-O_2+H_2+CO_2$ treatments. This CH_4, in turn, stimulated soil methanotrophy, resulting in increased rates of atmospheric CH_4 oxidation. Additions of CH_4 also increased atmospheric CH_4 oxidation, but to a lesser extent than $-O_2+H_2+CO_2$ treatments.

Homoacetogenic bacteria have requirements similar to methanogens, namely excess H_2 and CO_2, and strictly anaerobic conditions. West and Schmidt [27] showed that acetate additions can stimulate atmospheric CH_4 oxidation in these soils. We therefore used lumazine, a highly selective inhibitor of methanogens [19], to determine whether methanogenesis or acetogenesis had provided carbon for stimulation of atmospheric CH_4 oxidizers. Nagar-Anthal et al. [19] found that lumazine suppressed growth of all methanogenic species tested, but not of *Acetobacterium woodii,* a homoacetogen [19]. In this study, lumazine did not reduce atmospheric CH_4 oxidation (Table 4), indicating that lumazine also has minimal effect on atmospheric CH_4 oxidizers. In contrast, lumazine reduced soil CH_4 production by more than one-half (Table 4), confirming the effectiveness of lumazine at inhibiting methanogenesis even in a complex soil environment. Additions of lumazine prevented stimulation of atmospheric CH_4 oxidation by $-O_2+H_2+CO_2$ treatments (Fig. 4), confirming that methanogenesis was the more likely mechanism for stimulation of atmospheric CH_4 oxidation by anaerobic conditions with excess H_2 and CO_2 than was acetogenesis.

Anaerobic conditions created by N_2 flushing did not stimulate atmospheric CH_4 oxidation, probably because anaerobic conditions without excess H_2 and CO_2 did not stimulate CH_4 production in *Kobresia* soil. N_2-flushing actually suppressed CH_4 production in the *Carex* meadow soil (Table 3). Similarly, Moore and Dalva [18] found that CH_4 production under N_2-flushed headspace was inhibited relative to CH_4 production under aerobic headspace. N_2 flushing probably reduces CH_4 production by decreasing the amount of CO_2 normally available to soil methanogens, both by excluding the 350 ppm CO_2 normally present in the atmosphere and by excluding O_2 from the aerobic soil microbes that produce CO_2. Further studies would be necessary to confirm this explanation.

Methane production in *Kobresia* meadow soil under $-O_2+H_2+CO_2$ headspace was probably obscured by CH_4 consumption. During the treatment of the first experiment (Fig. 1), CH_4 concentrations in $-O_2+H_2+CO_2$ headspace were lower than in $+CH_4+O_2$ headspace, yet atmospheric CH_4 oxidation was twice as stimulated by $-O_2+H_2+CO_2$ as by $+CH_4+O_2$ treatment (Fig. 2). Therefore, methanotrophs in $-O_2+H_2+CO_2$ soils must have been exposed to more CH_4, which was consumed before entering the headspace of the microcosms. The feasibility of CH_4 consumption under anaerobic headspace is confirmed by the fact that N_2-flushed *Kobresia* soil consumed CH_4 at nearly 500 pmol g^{-1} h^{-1} (Table 2). Remnant oxygen in the palladium-scrubbed $-O_2+H_2+CO_2$ treatment is highly unlikely, but the methylene blue indicators that we used confirmed only that oxygen concentrations in both $-O_2+H_2+CO_2$ and N_2-flushed treatments were less than 0.5%. Enough oxygen may have remained for CH_4 oxidation to continue. Alternatively, anaerobic CH_4 oxidation may have been taking place, either by sulfate-reducing metabolism [6, 20] or by an unknown mechanism.

Methane consumption probably obscures CH_4 production in *Kobresia* meadow soil under aerobic headspace as well. Andersen et al. [1] found evidence that endogenous CH_4 production can occur simultaneously in soils that are consuming atmospheric CH_4. Such CH_4 production is the most likely mechanism by which wetting [26] and acetate [27] stimulate atmospheric CH_4 oxidation in *Kobresia* meadow soil. Wetting and acetate additions suppress atmospheric CH_4 oxidation before stimulating it, most likely because increased endogenous CH_4 production reduces net CH_4 consumption. Analogously, in Fig. 3, stimulation of CH_4 oxidation by $-O_2+H_2+CO_2$ treatment did not occur until more than 1 week after removal from anaerobic conditions, but CH_4 production in this soil rarely exceeds consumption (Fig. 3). Nevertheless, the atmospheric CH_4 oxidizers of *Kobresia* meadow soil appear to be dependent on this undetected CH_4 production. In all

experiments shown here (Figs. 2, 3, and 4) and in our previous studies [26, 27], atmospheric CH_4 oxidation in these soils slowly declined unless stimulated by wetting, methanogenic carbon additions, CH_4 additions, or $-O_2+H_2+CO_2$ treatments. In the field, atmospheric CH_4 oxidizers of these soils may be stimulated by temporary anaerobic conditions created by moisture additions such as after large summer rain events [28].

There is evidence for endogenous CH_4 production in other CH_4-consuming soils [1, 14, 30]. The concept of endogenous methane production in anaerobic soil microsites of CH_4-consuming soils has explanatory power. Endogenous CH_4 production would make superatmospheric concentrations of CH_4 locally available so that headspace CH_4 concentrations do not reflect what is available to soil methanotrophs. This may be why CH_4-consuming soils display higher-affinity uptake kinetics [2] than methanotrophic pure cultures [5] or enrichment cultures [10]. Endogenous CH_4 production would further explain why atmospheric CH_4 has varying importance in preserving atmospheric CH_4 oxidation [12, 25, 4]. It can also explain why varying quantities of headspace CH_4 are required to stimulate growth of atmospheric CH_4 oxidizers in various soils. In this study, adding 20 to 60 ppm headspace CH_4 for 6 days stimulated atmospheric CH_4 oxidizers (Fig. 2), but in previous studies, atmospheric CH_4 oxidation has not been stimulated by such low CH_4 concentrations [3, 25].

Conclusions

We demonstrated that anaerobic conditions with elevated H_2 and CO_2 stimulated atmospheric CH_4 oxidation in a dry alpine meadow soil. This effect was prevented by using lumazine to inhibit methanogenesis. These data demonstrate the importance of endogenous CH_4 production in a CH_4-consuming soil. Our current work is using molecular techniques to further elucidate the trophic relationship between methanogens and methanotrophs in these soils.

Acknowledgments

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