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Methane consumption in two temperate forest soils

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Abstract. Forest soils are thought to be an important sink for atmospheric methane. To evaluate methane consumption, ^{14}C-labeled methane was added to the headspace of intact soil cores collected from a mixed mesophytic forest and from a red spruce forest located in the central Appalachian Mountains. Both soils consumed the added methane at initially high rates that decreased as the methane mixing ratio of the air decreased. The mixed mesophytic forest soil consumed an average of $2\,mg\,CH_4\,m^{-2}\,d^{-1}$ versus $1\,mg\,CH_4\,m^{-2}\,d^{-1}$ for the spruce forest soil. The addition of acetylene to the headspace completely suppressed methane consumption by the soils, suggesting that an aerobic methane-consuming microorganism mediated the process. At both forest sites, methane mixing ratios in soil air spaces were greater than that in the air overlying the soil surface, indicating that these soils had the ability to produce methane. Models of methane emission from forest soils to the atmosphere must represent methane flux as the balance between production and consumption of methane, which are controlled by very different factors.

Introduction

Efforts to understand the observed 1% annual increase of tropospheric methane have focused primarily on increased emissions of methane from biogenic sources to the atmosphere (Cicerone & Oremland 1988 and references cited therein). A portion of the increase may also result from reduced strength of a methane sink, such as depletion of tropospheric hydroxyl radicals that remove atmospheric methane (Khalil & Rasmussen 1985; Isaksen & Hov 1987). Forest soils are also thought to be a sink of atmospheric methane (cf., Seiler & Conrad 1987). That is, field studies have shown that methane is lost from the headspace of chambers placed over soil surfaces for short time periods (Keller et al. 1983, 1986; Seiler et al. 1984). If all forest soils consumed atmospheric methane at the mean rate measured in these studies, the sink would be large enough to influence the global

atmospheric methane budget. However, few data have been collected and the mechanism involved is undefined.

Forest soils are mostly aerated, suggesting that aerobic methane-consuming microorganisms are likely to be present (Hanson 1980). Yet aerated forest soils can still have anaerobic microsites where oxygen consumption exceeds the rate of oxygen supply by diffusion (Smith 1980). These might be able to support active populations of anaerobic methane-producing bacteria, suggesting that methane fluxes are the balance between production and consumption.

In the studies reported here, soils from both a mixed deciduous forest and a red spruce forest located in the central Appalachian Mountains showed consumption of atmospheric methane as well as methane production. Thus, the balance between production and consumption of methane appears to determine whether a forest soil will be a source or sink of atmospheric methane.

Methods

Field site

Two forest stands in the Monongahela National Forest located in the Appalachian Mountain region of West Virginia were selected for study. One stand (39 °07′N, 79 °35′W; 990-m elevation) was a mixed mesophytic forest with American beech (*Fagus grandifolia* Ehrh.), red maple (*Acer rubrum* L.), yellow birch (*Betula alleghaniensis* Brit.), black cherry (*Prunus serotina* Ehrh.), and an occasional codominant eastern hemlock (*Tsuga canadensis* (L.) Carr.) established on an extremely stony, fine-loam, mixed, mesic Aquic Fragiuldult soil derived from Homewood sandstone. The other stand (38 °43′N, 79 °32′W; 1100-m elevation) was a monospecific red spruce (*Picea rubens* Sarg.) forest established on a loamy-skeletal, mixed, mesic Typic Dystrochrept soil also derived from Homewood sandstone.

The spruce forest had higher soil organic matter content and higher annual precipitation, which combined with lower evapotranspiration, contributed to greater soil moisture content compared to that in the mixed mesophytic forest.

Soil core collection

Five intact soil cores (10-cm dia × 25-cm depth) were obtained from each forest in May 1988 by driving separate PVC cylinders, each with a sharpened

lower edge, through the forest floor into the mineral soil. Each cylinder had a gas phase above the soil core to facilitate gas diffusion into and out of the soil. A PVC cap was placed on the top and bottom of each cylinder, and the intact soil cores were transported to the laboratory where each remained within a cylinder. On the day of collection, two soil cores from each forest were autoclaved for (>)20 min to inhibit microbial activity in the soil, two of the remaining soil cores were left undisturbed and the fifth soil core was extruded from its cylinder so that subsamples could be taken from each 5 cm depth interval for moisture content determination. The results reported here were typical of those determined on ten other cores taken between March and June 1988 in each forest.

Net methane flux measurements

The top cap on each cylinder had a septum to allow sampling of the headspace using a gas-tight syringe. Initially, 60 mL of gas were removed from each and replaced immediately with 60 mL of a stock ^{14}C methane prepared in nitrogen (0.5 μCi/mL). The addition was mixed by alternately drawing and releasing a vacuum on the syringe while the needle remained inserted into the cylinder headspace. Because the ^{14}C methane added to each cylinder had carrier unlabeled methane, the mixing ratio of methane in each cylinder was increased from ambient 1.80 ppmv to approximately 15 ppmv.

Periodically following the methane addition, a sample of each headspace was removed using a separate plastic syringe equipped with a Mininert on/off valve. One-half mL was injected into a Varian Model 6000 gas chromatograph fitted with a thermal conductivity detector. A Poropak R column (2-m long × 2.3-mm wide) was used with helium carrier at a flow rate of 30 mL min^{-1}. Oven and detector temperatures were 35 and 200 °C, respectively. The gas exiting the detector flowed directly to the input of a Packard Model 894 gas proportional counter. Make-up helium (20 mL min^{-1}) and propane quench gas (99.95%; 5 mL min^{-1}) were added to the gas stream, and gas flows were optimized for separation of ^{14}C methane and ^{14}C carbon dioxide on the gas proportional counter. The counting efficiency for ^{14}C was determined to be 50%. An additional 0.5 mL of each sample was injected into a different injection port on the gas chromatograph and detected by flame ionization.

The headspace of each cylinder was assayed for 15 h and immediately thereafter the distribution of ^{14}C activity in each cylinder was determined using methods described by Jones et al. (1982). First, the headspace was continuously flushed from the cylinder with nitrogen (10 mL min^{-1} for 4 h), and the effluent was passed through two gas traps placed in series; the first

contained 1 M sodium hydroxide solution to collect ^{14}C carbon dioxide, and the second contained toluene-based fluor to collect ^{14}C methane (cf., Zehnder et al. 1979). The relatively long flushing period was designed to remove gases in both the headspace and in soil air spaces. Next, the entire cylinder was frozen in liquid nitrogen. The frozen soil core was extruded and its volume was recorded (i.e., length times surface area). Triplicate 10 mg subsamples were sawn from each 5-cm depth interval of the frozen soil cores and transferred to individual 40-mL screw-capped vials and 10 mL of 1 M sodium hydroxide was added. After the sample thawed, a subsample of the vial headspace was taken and injected into the headspace of a scintillation vial that contained 12 mL of toluene-based flour to determine if any residual ^{14}C methane had remained in soil air spaces after the initial flushing; essentially none was detected. Two mL of the sodium hydroxide was added to 10 mL of scintillation cocktail to measure ^{14}C carbon dioxide that remained in soil airspaces. One ml of hyamine hydroxide in methanol was added to the remaining tissue sample in the vial to solubilize the organic matter (L'Annunziata 1979). The sample was solubilized for 20 h before 10 mL of scintillation cocktail was added and ^{14}C of the organic matter was determined. Scintillation counting was done on a Beckman liquid scintillation counter using the channels ratio method to determine counting efficiencies and quenching. The efficiency of all absorption procedures was checked with known quantities of ^{14}C methane and ^{14}C carbon dioxide and the results were corrected accordingly.

Acetylene addition

Acetylene is a powerful inhibitor of aerobic methane-consuming bacteria (Dalton and Whittenbury 1976), and we examined its effect on the mixed mesophytic forest soil. Eight intact soil cores were taken in June 1987. Unlabeled methane was added to each gas phase so that the initial mixing ratio was approximately 100 ppmv, and methane in the gas phase was monitored as a function of time until values reached approximately 50 ppm. At that point, four soil cores were randomly selected to receive 100 mL of acetylene, which was added after removing an equal volume of gas (i.e., C_2H_2 final partial pressure of 10 kPa). Each gas phase was thoroughly mixed, and monitoring of methane in the gas phase continued. Overall, each soil core assay lasted 10 h.

Methane in soil air spaces

Seven soil pits were located at 5 m intervals along an east-west oriented transect in each forest in June 1987. Each pit was permanently equipped

with five probes (see Seiler & Conrad 1981 for description) to sample methane in soil air spaces before the pit was backfilled. Probes at depths 2.5, 5, and 10 cm sampled the organic horizon, probes at the 20 cm depth sampled the E horizon and probes at the 50 cm depth sampled the B horizon. The top of each probe reached above the soil surface and was capped with a serum stopper. Soil-air samples were collected three times between May and July 1988 using 10-mL plastic syringes that also served as the storage vessel. Gas diffusion along the barrel of the syringe was inhibited by wetting the barrel with a small amount of distilled water, and the needle was capped with a silicon stopper. Samples were analyzed by gas chromatography within 24-h after collection.

Results

Intact soil cores from both forests consumed methane as soon as it was added to the cylinder headspace (Fig. 1). Thereafter, the rate of methane consumption appeared to decrease as the amount of methane in the headspace decreased. Towards the end of the incubation, the spruce forest soil maintained a mixing ratio of methane of approximately 10 ppmv which was substantially greater than 1.8 ppmv methane of ambient air, whereas the mixed mesophytic forest soil maintained less than 0.3 ppmv methane.

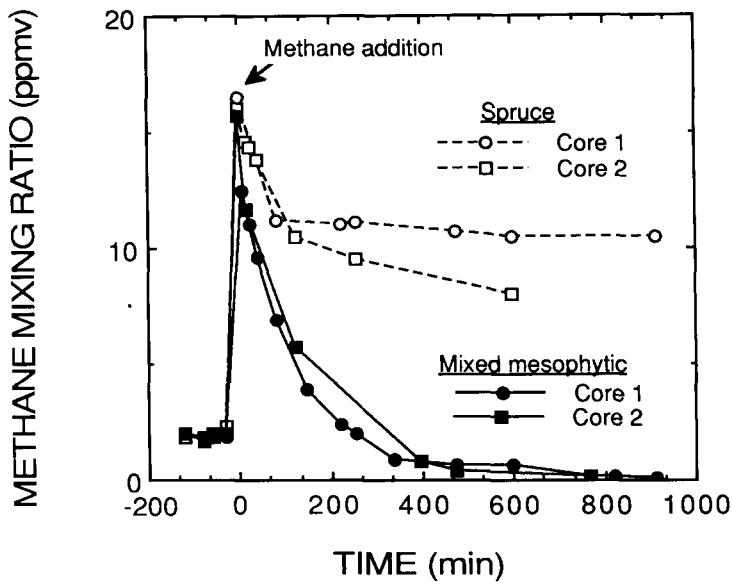

Fig. 1. Consumption of added methane by replicate soil cores from two different forest soils.

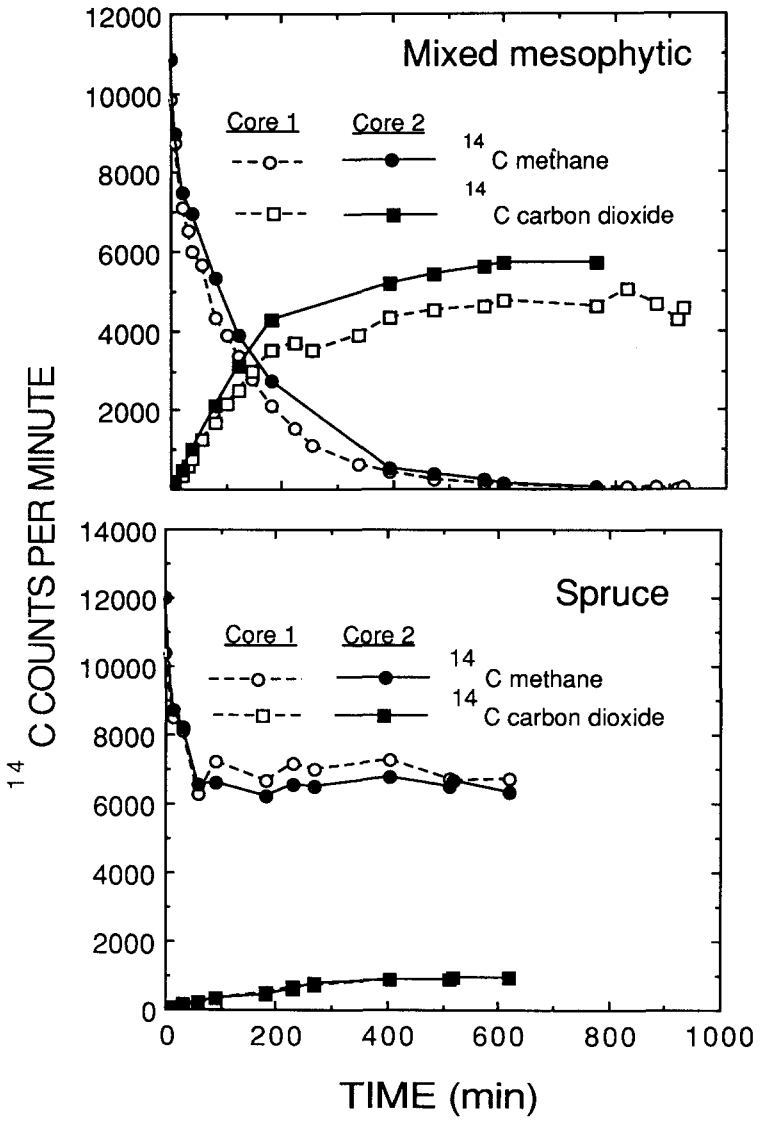

Fig. 2. $^{14}CH_4$ consumption and $^{14}CO_2$ production in replicate soil cores from two different forest soils.

Methane consumption was defined more clearly with added ^{14}C methane. For instance, the loss of ^{14}C methane was accompanied by ^{14}C carbon dioxide production (Fig. 2), indicating that at least a portion of the consumed methane was being oxidized to carbon dioxide. At the end of incubation, 70% of the ^{14}C added to the spruce forest soils was recovered in the flushed-out gas phase (headspace + soil air spaces); 80% as unreacted ^{14}C

methane and 20% as produced ^{14}C carbon dioxide. In contrast, only 50% of the added ^{14}C was recovered in the gas phase of the mixed mesophytic forest soils, and all was produced ^{14}C carbon dioxide. Thus, the mixed mesophytic forest soil appeared to consume more methane than the spruce forest soil.

The remaining ^{14}C activity was recovered in the soil organic matter fraction. For both soils, this fraction did not contain unreacted ^{14}C methane indicating that the ^{14}C activity of the soil organic matter was entirely products of methane oxidation. Unfortunately, we did not fractionate this ^{14}C activity to determine the relative proportions of soluble ^{14}C-labeled compounds that might have been excreted from methane-oxidizing microorganisms, ^{14}C-labeled methane carbon incorporated into microbial biomass, or ^{14}C carbon dioxide. Nevertheless, total recoveries of ^{14}C ranged from 97 to 103% (3.6% S.E., n = 4). Essentially complete recovery of the ^{14}C addition confirmed that gases were not leaking out of the cylinders which also would have given the appearance of methane consumption.

Twenty-five percent of the ^{14}C methane added to autoclaved forest soils was lost from the headspace, and all within the first few minutes of incubation (Fig. 3). Because methane loss was not accompanied by ^{14}C carbon dioxide production, the loss probably resulted from diffusion of methane into soil air spaces.

For the experiments with acetylene added to the gas phase, complete inhibition of methane consumption by the amended soils (Fig. 4) suggested that aerobic methane-consuming microorganisms played an important role.

In both forests, vertical profiles of methane using mixing ratios in soil air spaces showed values both enhanced and depleted with respect to air overlying the soil surface (Fig. 5). In the mixed mesophytic forest, for instance, maximum values of 2.00 to 2.50 ppmv at the 2.5 cm depth were greater than 1.9 ppmv of methane in the ambient air, suggesting in situ methane production in the surface soil layer. In contrast, zones of methane consumption apparently occurred 10 and 25 cm below the surface where methane mixing ratios were as low as 1.35 ppmv. Differences among sampling dates were not significant for methane in soil air spaces and for soil moisture contents (45 and 30% soil moisture at 0 to 10 cm depth and 10 to 30 cm depth, respectively).

Methane in air spaces in the spruce forest soil was variable among the three sampling dates. For instance, the lowest values occurred on May 13, and soil moisture contents on this date were less than 40% throughout the soil profile. High methane values on June 3 were associated with high soil moisture contents of 60 to 70% throughout the soil profile. Intermediate values for both parameters occurred on July 19 (60 and 30% soil moisture

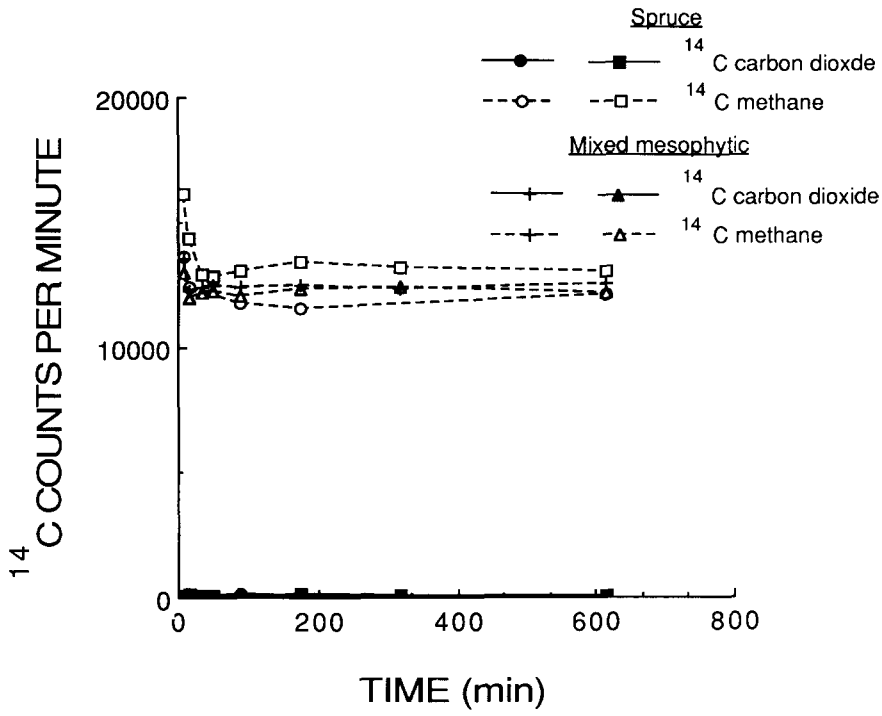

Fig. 3. $^{14}CH_4$ consumption and $^{14}CO_2$ production in autoclaved soil cores from two different forest soils.

at 0 to 10 cm depth and 15 to 20 cm depth, respectively). These patterns suggest that differences in soil moisture may explain at least a portion of the variation of methane content of soil air.

Discussion

Rates of methane consumption were calculated from the decreasing slope of the methane mixing ratio shown in Fig. 1. For both forest soils, the decrease appeared to fit an exponential rate of decline, suggesting that methane consumption was first order with respect to the concentration of methane in the gas phase (cf., Conrad 1984). First-order kinetics have been confirmed for methane consumption by pure cultures of methane-consuming bacteria in laboratory studies (Harrison 1973); however, confirmation of nonlinearity *in situ* requires more detailed experimental studies using different amounts of methane added to intact soil cores. Consequently, with the data we had available, we calculated rates of methane consumption for different

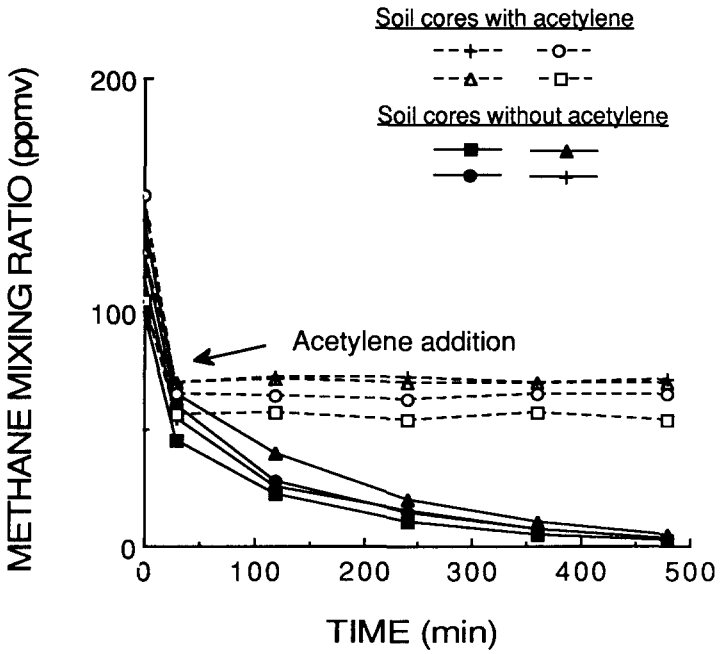

Fig. 4. Effect of acetylene on methane consumption by soil cores from a mixed mesophytic forest.

mixing ratios of methane by fitting a straight line to the methane values between two different time periods.

The results showed that methane consumption rate of these soil varied by an order of magnitude for mixing ratios of methane from 15 to 1.8 ppmv. For instance, during the first 120 min of the assay period and with an elevated mixing ratio of methane, calculated rates of methane consumption were -4.9 and $-10.8\,\mathrm{mg\,m^{-2}\,d^{-1}}$ for the spruce forest soil and the mixed mesophytic forest soil, respectively. However, between 200 and 400 min and with about 1.8 ppmv methane, the mixed mesophytic forest soil consumed $0.6\,\mathrm{mg\,CH_4\,m^{-2}\,d^{-1}}$. This value was in reasonable agreement with published values obtained from field studies of methane consumption by tropical and temperate forest soils ($0.2\,\mathrm{mg\,m^{-2}\,d^{-1}}$ Keller et al. 1983; $1.2\,\mathrm{mg\,m^{-2}\,d^{-1}}$ Seiler et al. 1984). Thus, our laboratory measurements appear to be similar to field measurements at comparable mixing ratios of atmospheric methane.

Because the experiments were initiated at higher concentrations of methane than usually found in air, it is possible that methane was saturating methane-consuming microorganisms. Therefore, a pertinent question was how much of the calculated methane consumption at an elevated mixing ratio was microbially mediated? The results from the autoclaved soils helped

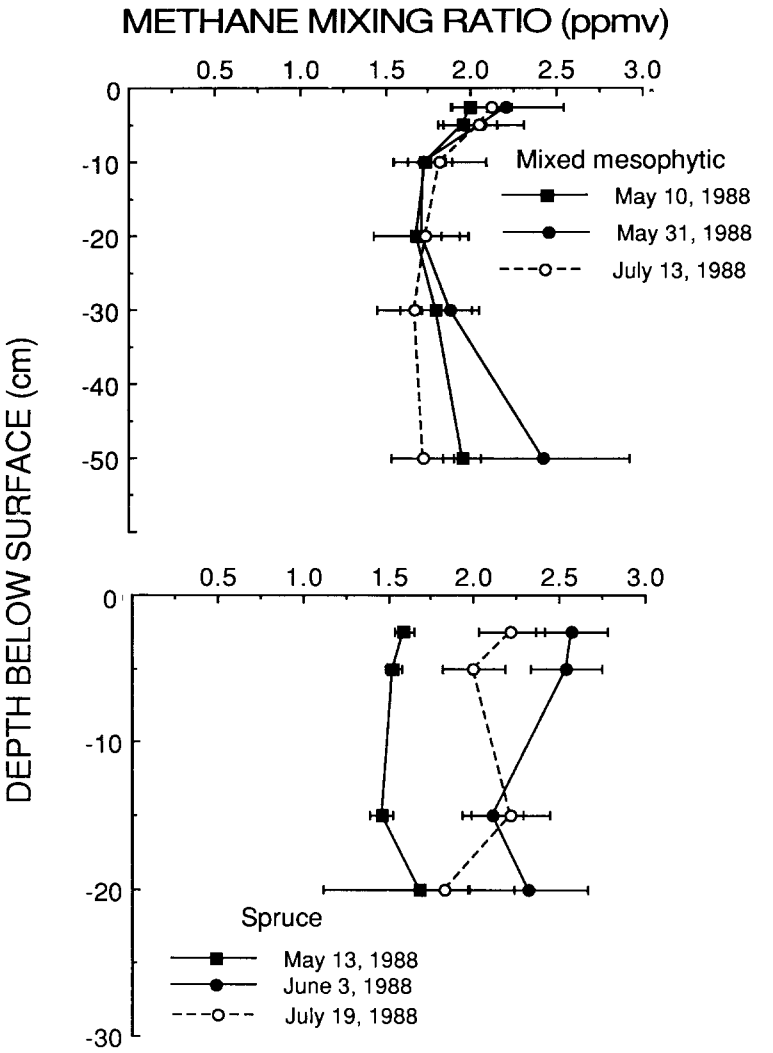

Fig. 5. Profiles of methane mixing ratios in soil air spaces for two different forest soils. Methane mixing ratio in ambient air above the surface of both soils (50 cm height) was 1.95 ppmv (0.20 SE, n = 10). Error bars represent + 1 SE (n = 7).

evaluate this question. In these soils, microbially mediated methane consumption was inhibited. Thus, the 25% methane loss shown in Fig. 3 must have been supported by diffusion into the soil as a result of a concentration gradient across the soil-air interface. Using this correction, 25% of the initial methane consumption shown in Fig. 1 could have been supported by diffusion alone and without intervention of microorganisms. The result can

account for change of the methane mixing ratio from 15 to 11.2 ppmv. Coincidentally, 10 ppmv was the equilibrium mixing ratio of methane maintained by the spruce forest soil, and also the point where oxidation of the ^{14}C-methane addition ceased, suggesting that methane consumers in these soils were not able to deplete the concentration of atmospheric methane below 10 ppmv. Nevertheless, the production of ^{14}C carbon dioxide confirmed that the consumer population was active.

Although the spruce forest soils consumed atmospheric methane, they would act as a sink only at elevated mixing ratios of atmospheric methane. Elevated values can occur during local atmospheric inversions, provided that there is a methane source in the area. That is, methane from the source can become trapped below the boundary air layer, thus preventing escape to the upper troposphere (cf., Gurney et al. 1988). One of us (J.B.Y.) has measured 40 ppmv methane below an atmospheric inversion layer of the study area where a moss-dominated wetland adjacent to the forest was the source of atmospheric methane (J.B. Yavitt, pers. observ.).

In contrast, the mixed mesophytic forest soil depleted methane in the headspace to 0.3 ppmv (Fig. 1), making this soil a potential sink for atmospheric methane even at ambient concentrations of atmospheric methane. However, for the soil to have continued to consume methane at such a low concentration of atmospheric methane, methane in soil air spaces must have been less than 0.3 ppmv to support a concentration gradient across the soil-air interface. This was not suggested by measurements in the field (Fig. 5), but those data were limited to only three sampling dates.

Whether the mixed mesophytic forest soils act as a sink of atmospheric methane in the field depends on methane mixing ratios in air and in soil air spaces. Flux of atmospheric methane into the soil was evaluated using Fick's relationship of diffusion:

$$J = D_F dC/Dz,$$

where J is the mass transfer rate ($mg\,cm^{-2}\,s^{-1}$), D_F is the Fickian diffusion coefficient ($0.186\,cm^2\,s^{-1}$ for methane in air, Marrero & Mason 1972), C is the concentration ($mg\,cm^{-3}$), and z is the distance (cm). Thus, assuming that a local air mass above the forest soil has only 2.25 ppmv methane and using the data in Fig. 5 for methane in soil air spaces, the calculated flux of methane into the soil would be $-2\,mg\,m^{-2}\,d^{-1}$. This flux would make the soils a small but measurable sink of atmospheric methane.

Methane consumption by these soils does not preclude methane production. For instance, methane values in soil air spaces that were greater than the atmospheric value (Fig. 5) could have occurred only via a net methane

production in the soil. Furthermore, Sexstone and Mains (1989), using in vitro experiments, estimated potential production of 1×10^6 ml CH_4 g^{-1} soil h^{-1} in the uppermost soil horizon of the spruce forest soil, indicating the existence of an active population of anaerobic methane-producing microorganisms in these generally aerated soils.

Certainly different factors would favor consumption versus production of methane in a forest soil. For instance, methane production should dominate with increasing soil moisture, whereas methane consumption should dominate with increasing aeration of the soil. Although moisture enhances conditions for anaerobic methane-producing bacteria, it should be recognized that increased methane production does not linearly equate with increased emission of methane from the soils to the atmosphere. Increasing soil moisture may also block air spaces preventing methane diffusion between the atmosphere and soil air spaces. Both Conrad & Seiler (1985) and Galbally & Johansson (1989) have shown recently that even slight changes in soil moisture can have large impacts on net fluxes of atmospheric trace gases by surface soils through effects on microbially mediated production and consumption of gases and/or diffusionally supported flux across the soil-air interface.

We evaluated the source strength of these soils for atmospheric methane using the simple diffusion model. With methane values in the air and in soil air spaces shown in Fig. 5, calculated methane diffusion from the soil to the atmosphere would have been 10 mg $m^{-2}d^{-1}$. Field studies with chambers also have shown events of methane emission from the mixed mesophytic forest soils to the atmosphere, with values between 0.5 and 40 mg $m^{-2}d^{-1}$ (J.B. Yavitt, pers. observ.), thus making these soils sources of atmospheric methane, at least under certain conditions.

From these studies, we concluded that the spruce and mixed mesophytic forest soils showed both production and consumption of methane, potentially giving them the ability to be both sources and sinks of atmospheric methane. Because each process is controlled by different factors, these must be understood for models (cf., Cicerone & Oremland 1988) that incorporate the effect of forest soils on the global atmospheric methane budget.

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