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Effects of Temperature on Methane Consumption in a Forest Soil and in Pure Cultures of the Methanotroph *Methylomonas rubra*†

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Methane oxidation in soil cores from a mixed hardwood-coniferous forest varied relatively little as a function of incubation temperatures from −1 to 30°C. The increase in oxidation rate was proportional to $T^{2.4}$ (in kelvins). This relationship was consistent with limitation of methane transport through a soil gas phase to a subsurface zone of consumption by diffusion. The Q_{10} for CO_2 production, 3.4, was substantially higher than that for methane oxidation, 1.1, and indicated that the response of soil respiration to temperature was limited by enzymatic processes and not diffusion of either organic substrates or molecular oxygen. When grown under conditions of phase-transfer limitation, cultures of *Methylomonas rubra* showed a minimal response to temperature changes between 19 and 38°C, as indicated by methane oxidation rates; in the absence of phase-transfer limitations, *M. rubra* oxidized methane at rates strongly dependent on temperature.

The primary biological sink for atmospheric methane is soil. Net uptake by other systems has been reported only from the Bering Sea shelf, although methane oxidation does limit fluxes from lakes, wetlands, rice paddies, and landfills (20). Net oxidation of atmospheric methane was first reported by Harriss et al. (11), who observed methane uptake as the water table dropped several tens of centimeters below the surface of sediments in the Great Dismal Swamp. Subsequently, Keller et al. (16–18) reported atmospheric methane oxidation by both temperate and tropical forest soils; Seiler et al. (25) observed net uptake by tropical savannah soils associated with termite mounds. More recently, atmospheric methane oxidation has been documented for a wide variety of soils (3, 21, 23, 30, 31), and some studies have suggested possible controls of the process that are related to nitrogen dynamics (23, 27). On the basis of the various rates measured thus far, global soil methane oxidation ranges between 5 and 50 Tg $year^{-1}$ (3, 8). These values are approximately 1 to 10% of the current estimates for net methane flux to the atmosphere (8). While current rate estimates do not support an important role for soil oxidation in the increase of atmospheric methane, it is not at all certain how large-scale changes in land use and climate will affect the contribution of this process to methane budgets in the future. For example, some agricultural practices might lower oxidation rates, while climate changes could have either positive or negative effects.

Predictions of future trends in atmospheric methane oxidation by soils clearly require a much greater understanding of the basic microbiology and controls of the process. At present, there is even uncertainty about the ability of methanotrophs to grow while oxidizing atmospheric methane (5). The evidence for uptake by ammonia-oxidizing bacteria is also equivocal (2, 15, 20, 28, 29). Soil moisture clearly plays an important regulatory role (31), but the significance of other factors remains vague. The response of soil methane oxidation to temperature merits specific attention. Oxidation is known to occur from about 4 to 25°C, but the nature of temperature regulation across this range has not been addressed in detail. Born et al. (3) have reported little seasonal variation in atmospheric methane oxidation by forest soils but did not examine temperature effects per se. Whalen et al. (31) found a distinct temperature optimum (31 to 36°C) for landfill soils, but methane concentrations were about 5,000-fold greater than atmospheric values. Crill (7) observed a variable response of methane oxidation to temperature as a function of seasonality; the response to increasing temperature was strongest in the spring and more damped in the summer. It is important to clarify the reasons for these varying responses since soil methane oxidation occurs primarily in latitudes with substantial variations in seasonal temperatures; these latitudes will likely experience significant future temperature fluctuations as a result of global warming.

MATERIALS AND METHODS

The forest soils used in this study were collected from a mixed hardwood-coniferous forest adjacent to the Darling Marine Center, Walpole, Maine. The soils were oxic podzols (oxygen content, about 21% at a depth of 0 to 12 cm) with a pH of about 4 and a water content which decreased sharply from 180% (in grams [gram dry weight]$^{-1}$) in the organic horizons to 40% in the mineral horizon. Soil temperatures in June 1991 decreased from 22 to 14°C in the upper 3 cm and remained more constant at greater depths. The upper 7 cm encompassed the "O" organic horizons, with total carbon contents of >50% (wt/wt) determined by elemental analysis (Carlo Erba 1100); soils at depths of >7 cm were primarily mineral in nature, with total carbon contents of only 5%.

Soils were collected in acrylic tubes (30 cm by 63 mm [inside diameter]) by carefully cutting through the organic horizon around the circumference of the tubes with a serrated knife and thereafter gently twisting and pushing the tubes until 12 to 15 cm of soil was enclosed. Compression of

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the soils was <5%. A headspace of about 15 cm was left above the soils after sealing the core tubes with green neoprene stoppers. Immediately after collection, the cores were placed in a water bath with about 5 cm of the total core length above the water surface. The temperature difference between the headspace and soil was <5°C (temperatures indicated in the text and figures are those for the soil).

The response of atmospheric methane uptake to temperature was analyzed for a single set of five cores over a range of −1 to 30°C. Temperature was increased initially in a stepwise manner from 2 to 30°C and then returned to 2°C; subsequently, temperature was decreased stepwise to −1°C. Before uptake rates were determined, soil cores were equilibrated at each incubation temperature for ≥8 h, with the core headspace open to the laboratory atmosphere. Ambient methane concentrations in the laboratory atmosphere were analyzed routinely, and experiments were initiated only when the concentrations were ≤1.7 ppm. After the cores were carefully sealed to prevent overpressurization, headspace samples were collected with 1-ml disposable syringes and needles for methane analysis with a Shimadzu 14A gas chromatograph and a flame ionization detector operated at 150°C. Methane was separated with a Porpak Q column (1 m by 3 mm [outside diameter]) in series with a wide-bore capillary column (DB-1; 30 m by 0.53 mm [outside diameter]; J&W Scientific, Inc.). The nitrogen carrier flow rate was 20 ml min^{-1} at 40°C. The detector response was standardized with certified master gas standards (Scott Specialty Gases) containing either 0.861 ppm (+2%) or 100.4 ppm (+1%) methane in nitrogen. The detection limit for methane was 0.1 ppm. The coefficient of variation for replicate injections of the 0.861 ppm standard was 4%. CO_2 was analyzed by a Varian 3700 gas chromatograph with a thermal conductivity detector operated at 250°C. CO_2 was separated in a stainless-steel column (3 m by 3 mm [outside diameter]) containing Porpak Q. The helium carrier gas flow rate was 20 ml min^{-1} at 50°C. The detector response was standardized by diluting 100% CO_2 to final concentrations of 4,470, 8,630, and 12,800 ppm.

Methane uptake rate constants were calculated by using a linear regression of logarithmically transformed concentration data from the headspace since uptake was a first-order process. Rates of CO_2 production were analyzed by a simple linear regression since concentrations increased in direct proportion with incubation time.

The effect of temperature on methane oxidation by the type I methanotroph *Methylomonas rubra* was examined by using replicate cultures incubated with either high or low methane concentrations. Cultures were grown in a medium containing the following (in grams $liter^{-1}$): $NaNO_3$, 0.85; K_2SO_4, 0.17; $MgSO_4 \cdot 7H_2O$, 0.037; $CaCl_2 \cdot 2H_2O$, 0.007; KH_2PO_4, 0.53; Na_2HPO_4, 0.86. This medium was supplemented (per liter) with 2 ml of a solution containing the following (in grams $liter^{-1}$): $ZnSO_4 \cdot 7H_2O$, 0.287; $MnSO_4 \cdot 7H_2O$, 0.223; H_3BO_3, 0.062; $NaMoO_4 \cdot 2H_2O$, 0.048; $CoCl_2 \cdot 6H_2O$, 0.048; KI, 0.083; $CuSO_4 \cdot 5H_2O$, 0.125. The medium also contained (per liter) 1 ml of a solution of $FeSO_4 \cdot 7H_2O$ (11.2 g $liter^{-1}$), which was filter sterilized and added after autoclaving. The final pH of the medium was 7.0. Five hundred milliliters of medium inoculated with *M. rubra* was incubated with 10% methane–1% CO_2 and gentle shaking at 28°C. After reaching the stationary phase, the culture was harvested by centrifugation and washed twice with fresh medium. Ten-milliliter volumes of cells resuspended in fresh medium at a cell density of approximately 10^9 ml^{-1} were pipetted into sterile 120-ml serum bottles that were subsequently sealed with butyl serum stoppers. After addition of sufficient ultra-high-purity methane to produce final concentrations of about 100 or 10,000 ppm, the bottles were equilibrated for 1.5 to 3 h at temperatures from 5 to 45°C while shaking on a rotary shaker at 150 rpm. Methane concentrations in the headspace were determined by removing 0.3-ml subsamples at intervals by using a needle and syringe and the chromatographic system described previously. Uptake rate constants were calculated for duplicate bottles from a linear regression of logarithmically transformed data since uptake was first order at 100 ppm. Uptake rates in triplicate bottles containing about 10,000 ppm methane were calculated from a simple linear regression since consumption was zero order at high concentrations.

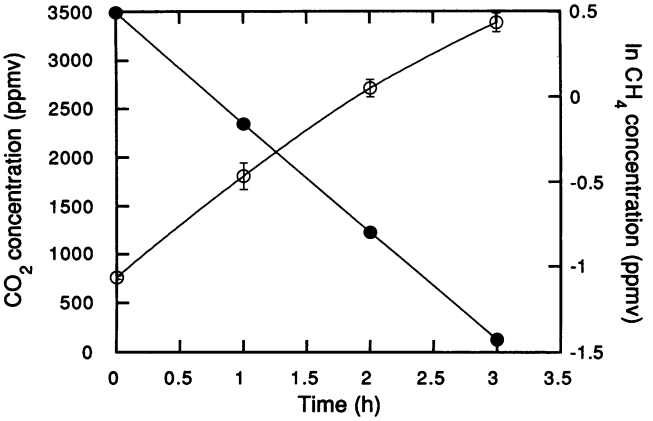

FIG. 1. Methane consumption (●) and CO_2 (○) production in soil cores incubated at 15°C. Each point is the mean of quintuplicate analyses; vertical bars represent ±1 standard error.

RESULTS

During the incubation periods at all temperatures, methane concentrations in the core headspaces decreased exponentially (Fig. 1), reaching concentrations of approximately 0.1 ppm after 7 h. The calculated rate constants varied from about 0.20 to 0.73 h^{-1} over a temperature range of −1 to 30°C (Fig. 2); oxidation rates estimated from the observed rate constants for a 1.7-ppm atmospheric methane concentration varied from 0.9 (±0.8) to 3.2 (±0.3) mg of methane m^{-2} day^{-1} (means ± standard errors; $n = 5$). The increase in rate constants from 0 to 30°C represented a total change of about 35%. The change in rate constants with temperature was described by a power function, $k = aT^b$, where b is 2.4 ± 1.1 (95% confidence interval). The response of the cores to temperature was apparently not affected by the length of time over which the experiment was conducted (32 days); the rate constant determined initially at 2°C was identical to the rate constant obtained at the same temperature 10 days later.

An entirely different pattern was observed for CO_2 production. CO_2 accumulated in the core headspaces at all temperatures (Fig. 1), with rates varying markedly as a function of incubation temperature (Fig. 3). The ratios of CO_2 production to methane consumption ranged from about 120 to 2,300 for 2 to 30°C, respectively. Consequently, methane consumption accounted for a variable but mostly negligible fraction of the soil carbon dynamics. Q_{10} estimates also differed markedly for methane consumption and CO_2 production. Values derived from the curve fits for rate

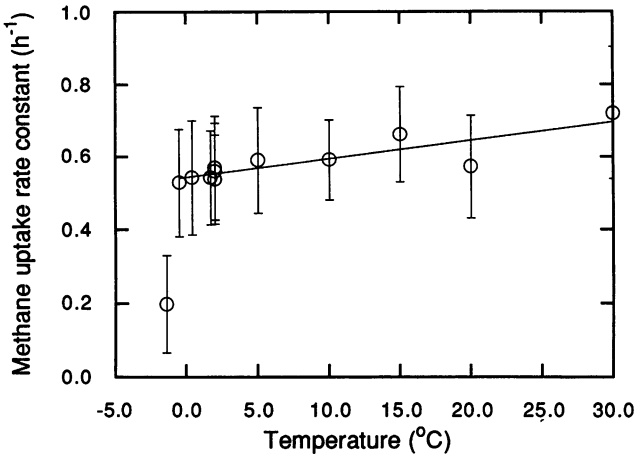

FIG. 2. Rate constants for methane oxidation versus incubation temperature; each point is the mean of quintuplicate analyses; vertical bars represent 95% confidence intervals obtained from curve fits for uptake data presented in Fig. 2.

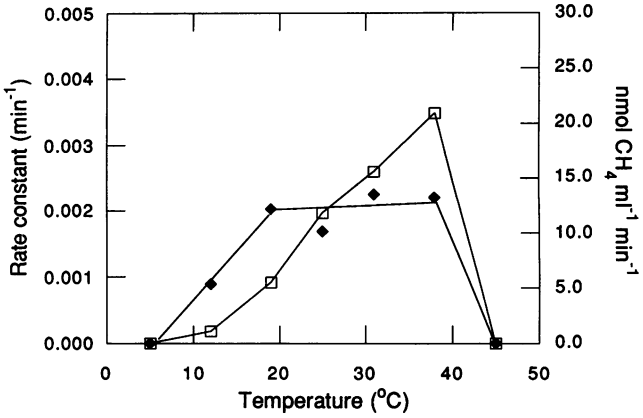

FIG. 4. Methane consumption by cultures of *M. rubra* as a function of temperature and methane concentration. Uptake rate constants for cultures incubated with 100 ppm methane (◆) are the means of duplicate analyses; uptake rates for cultures incubated with 10,000 ppm methane (□) are the means of triplicate analyses.

versus temperature were 1.1 and 3.4 for methane consumption and CO_2 production, respectively.

In cultures of *M. rubra*, the response to temperature depended in part on the headspace methane concentration to which cells were exposed (Fig. 4). At low methane concentrations (e.g., 100 ppm), the response was apparently first order and the uptake rate constants varied little between 19 and 38°C, with no distinct optimum. In addition, rate constants decreased to 0 at temperatures of 5 and 45°C. At higher concentrations (e.g., 10,000 ppm), methane uptake was zero order and a sharp, consistent increase in uptake rate was observed from 12 to 38°C; a distinct optimum occurred at 38°C, with rates falling to 0 at 5 and 45°C (Fig. 4). The maximum uptake rate corresponded to a value of about 10^{-17} mol $cell^{-1}$ min^{-1}. The activation energy calculated for the results from the 10,000-ppm methane concentrations was 52.7 kJ $kelvin^{-1}$ mol^{-1}.

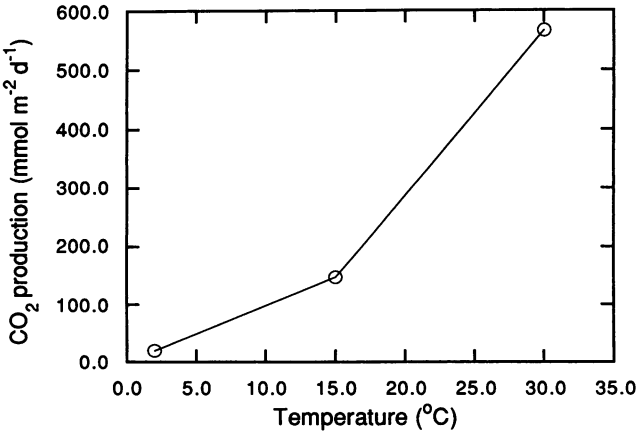

FIG. 3. CO_2 production rates versus temperature for the same soil cores used for the methane oxidation analyses summarized in Fig. 2. Each point is the mean of quintuplicate analyses; error bars are within the symbols.

DISCUSSION

While methane oxidation in freshwater ecosystems has been reviewed in some detail (20), the oxidation of atmospheric methane has been addressed only recently. To date, the net oxidation of atmospheric methane appears to be primarily restricted to, but ubiquitous within, soil ecosystems (e.g., 3, 7, 23, 27, 30–32). Neither the microorganisms responsible for nor controls of soil methane oxidation are known unequivocally. Soil water, nitrogen content, and temperature have been suggested as important controls (e.g., 7, 23, 27, 30, 31), but there is some uncertainty about the mechanisms by which these parameters regulate activity. For example, temperature reportedly has variable effects. Steudler et al. (27) and Born et al. (3) found little or no effect of temperature changes during seasonal studies of methane consumption, while Crill (7) has reported moderate effects during spring and more minimal effects during summer. The seemingly minor role of temperature as a regulatory factor for atmospheric methane oxidation differs substantially from the usually critical role it plays for other microbial processes.

The effects of temperature may be explained by examining the vertical distribution of soil methane oxidation and the mechanisms involved in methane transport. Crill (7) reports that methane consumption is highly stratified, at least in forest soils (7), with a pronounced subsurface maximum and little or no uptake in the uppermost horizons. In the soils used for this study, methane consumption occurs primarily in a zone at a depth of approximately 6 to 8 cm, with little or no consumption above and below this horizon (1). While reasons for such a stratification are not clear, the active horizon is not immediately adjacent to the source of methane, i.e., the atmosphere.

The transport of gas, such as methane, within the soil matrix to the zone of uptake is dominated by molecular diffusion as described by Fick's First Law (for an example, see reference 19). Within the zone of consumption, the rate of methane transport from the gas to the soil liquid phase can be approximated by:

$$J_L = -K_{(T)w} dC/dx \quad (1)$$

where $K_{(T)w}$ is the transfer velocity (directly proportional to the molecular diffusion coefficient, D, for nonreactive gas-

TABLE 1. Root-mean-square (RMS) diffusion times for various aqueous film thicknesses, calculated by using equation 2[a]

Film thickness (μm)	RMS diffusion time (s)	Soil water (%)[b]
0.1	3×10^{-6}	10
1.0	3×10^{-4}	100
10.0	3×10^{-2}	1,000

[a] The diffusion coefficient for methane in water is 1.49×10^{-5} cm^2 s^{-1} at 298 K (9).

[b] Calculated for an assumed surface area of 1 m^2 [gram dry weight]$^{-1}$ and expressed as weight of water [gram dry weight] of soil^{-1} × 100%.

es), dC/dx is the concentration across a stagnant, thin-film aqueous diffusive boundary layer of thickness dx, and dC is $(C_{air}H^{-1} - C_{aq})$, with C_{air} and C_{aq} being the gas- and liquid-phase concentrations, respectively, and H being the Henry's Law Constant (10). Excluding temperature-related variations in the term dC/dx, the variation of both gas-phase transport and J_L as a function of temperature can be described by the Chapman-Enskog theory and the Stokes-Einstein equation (or related variants), respectively (9). In the former, the gaseous diffusion coefficient varies as $T^{1.5}/\Omega$ with T in kelvins; the collision integral, Ω, has values near 1 for conditions of interest in soils. The Fuller correlation also describes gaseous diffusivity as a function of T; in this case, $D \sim T^{1.75}$ (9). In dilute liquid media, where the Stokes-Einstein equation is applicable, $D \sim T\,\eta^{-1}$, where η is viscosity. A simple calculation reveals that diffusion coefficients for gaseous methane change by about 21% over the range of 0 to 30°C and by about 150% over the same temperature range for dissolved methane.

In contrast, the relationship between temperature and enzymatically controlled processes (e.g., methane oxidation) is more complex. In general, however, enzymatic activity varies parabolically as a function of temperature, with a distinct maximum, minimum, and optimum; the region between the minimum and optimum is often described by the Arrhenius relationship.

Understanding the response of soil methane oxidation to temperature thus becomes a matter of understanding which factor is rate limiting: transport in the gas phase, gas exchange with soil water, or enzyme-based activity. The absence of a typical parabolic behavior suggests that enzymatic processes are not usually rate limiting. Calculation of the time constants for gas-phase transport and soil water-gas exchange facilitates an assessment of the relative importance of these two factors. A root-mean-square diffusion time can be calculated from the following relationship:

$$t = x^2/2D \quad (2)$$

where t is time and x is the diffusion distance. For the soil used in this study, the diffusion time for the gas phase is about 10.7 min (Table 1) when a depth of 7 cm is used as the zone of consumption (1). Comparable consumption depths have been reported for other soils (3, 7). Diffusion times for gas-soil water exchange vary depending on the choice of x; however, film thicknesses of even 400 μm yield values of ≤1 min (Table 1). Since a uniform distribution of water over the surface area of typical soils (1 to 10 m^2 g^{-1}) (13) results in thicknesses ≪400 μm, it is reasonable to conclude that methane oxidation is limited primarily by methane transport in the soil atmosphere.

Such a conclusion is consistent with the temperature response reported here (Fig. 2) and the responses reported by others (3, 7, 27). For the soil cores used in this study, the variability of rate constants as a function of temperature is described as $T^{2.4 \pm 1.1}$. This coefficient for temperature does not differ statistically from that predicted by the Fuller correlation or the Chapman-Enskog theory (e.g., 1.8). The tendency for a value higher than either of these coefficients can be attributed to experimental error and contributions from microbial activity or temperature-viscosity effects on liquid diffusion.

Data from Whalen et al. (31) and Crill (7) provide additional insights about the sensitivity to and rate-limiting steps for soil methane oxidation. Whalen et al. (31) have shown a typical enzymatic response for methane oxidation over the range of 5 to 46°C in landfill soils exposed to methane concentrations of about 10,000 ppm. At these high concentrations, methane consumption becomes saturated and the rate-limiting step is no longer gas-phase transport or gas-soil water exchange but enzymatic activity. Consequently, the temperature response is approximately parabolic.

Crill (7) has shown that methane consumption in forest soils changes by only 40% from June to December over an ambient air temperature range of −4 to 21°C; smaller changes occur over a range of 11 to 21°C from June to October. These observations agree well with the 30% change over the range of 0 to 30°C described here for intact cores analyzed in June. However, Crill reports that methane oxidation increased by a factor of 2.3 over a range of 7 to 19°C from April to June. The response during summer and fall, when the soils are relatively dry, is consistent with a gas-phase transport limitation. In contrast, the response during spring, when soils are relatively wet, suggests that limitations due to microbial activity at low temperatures and restricted diffusion resulting from decreased porosity (1, 7, 31) become more important than gas-phase transport. Thus, the sensitivity of soil methane consumption to temperature reflects a combination of variables, including soil water content, gas-phase methane concentrations, the depth of the methane-oxidizing horizon, and the population densities of methanotrophs.

In contrast, CO_2 production is strongly sensitive to temperature (for an example, see reference 7) (Fig. 3). The observed Q_{10}, 3.4, is comparable to values reported by Crill (7) and consistent with the response of a process determined by the behavior of enzyme systems. Since the soil microflora live in close association with organic substrates, a response comparable to that of methane oxidation would only be expected if oxygen transport limited activity. Such a limitation is not likely.

Results from studies with *M. rubra* also illustrate the significance of transport and physiological phenomena on the temperature response of methane oxidation (Fig. 4). Cultures incubated with both 100- and 10,000-ppm concentrations oxidize no methane at either 5 or 45°C; at both concentrations, the response to temperatures between 5 and 19°C appears to involve a switch-on phenomenon, perhaps indicating that methane oxidation is controlled by nonlinear effects of temperature on one or more key enzyme systems. At 100 ppm, little additional response to temperature occurs from 19 to 38°C. This is explained by a phase-transfer limitation (24) resulting from relatively high cell densities and low methane concentrations. During phase-transfer limitation, oxidation rates are limited by exchange between the liquid and gas phases, not biological activity, and a relatively small temperature response is expected. In contrast, at 10,000 ppm, methane oxidation was not phase-transfer lim-

ited but was enzymatically limited. Consequently, a pronounced temperature response is expected.

The culture data are also notable in that no methane oxidation occurred at 5°C. This is not an unusual observation for cultures, since many isolates do not grow at <10°C (for examples, see references 12, 14, and 26), even though temperatures in the habitats from which the isolates originated drop below these values. What is striking, however, is that numerous studies document active methane oxidation in situ at <5°C. In this study, methane consumption was even observed at −1°C; in fact, consumption may occur at lower temperatures as long as the soil water remains liquid. Thus, one is forced to question the extent to which the characteristics of existing isolates, or attributes observed at elevated temperatures, reflect the properties of methanotrophs active in situ. Perhaps as-yet-unisolated psychrophiles or psychrotrophs dominate soil methane oxidation.

The relationship between temperature and soil methane consumption reported here is apparently not unique. Liebl and Seiler (22) and Conrad and Seiler (6) have indicated that temperature has relatively small effects on CO oxidation. Similar relationships may occur for the uptake of other gases, such as hydrogen and nitrous oxide, especially if uptake is restricted to depths some distance from the soil surface.

CO_2 production in forest soils and, by inference, oxygen uptake display a rather different relationship (Fig. 3) (3, 7). This is probably due to the fact that organic matter mineralization is distributed throughout the soil profile and that oxygen uptake is saturated at the high partial pressures typical of aerated soils; under such conditions, enzymatic activity and not diffusive transport becomes rate limiting. Enzymatic control is consistent with reported Q_{10} values of 2.9 (7), 3.4 (this study), and 4.3 (3).

In summary, soil methane consumption is much less sensitive to changes in temperature than other biological processes, including bulk soil respiration. The lower sensitivity is explained by the depth distribution of consumption and the mechanism of methane transport (i.e., diffusion) and to a lesser extent by gas-liquid exchanges and enzymatic processes. This modest temperature sensitivity suggests that rates of soil methane consumption may not be directly affected by changes in global temperature per se. Changes in water regimens and atmospheric methane concentrations are probably of much greater significance as rate determinants.

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