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Responses of Methanotrophic Activity in Soils and Cultures to Water Stress†

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Diffusive gas transport at high water contents and physiological water stress at low water contents limited atmospheric methane consumption rates during experimental manipulations of soil water content and water potential. Maximum rates of atmospheric methane consumption occurred at a soil water content of 25% (grams per gram [dry weight]) and a water potential of about −0.2 MPa. In contrast, uptake rates were highest at a water content of 38% and a water potential of −0.03 MPa when methane was initially present at 200 ppm. Uptake rates of atmospheric and elevated methane decreased when water potentials were reduced by adding either ionic or nonionic solutes to soils with a fixed water content. Uptake rates during these manipulations were lower when sodium chloride or potassium chloride was used to adjust water potential rather than sucrose. The response of methane consumption by soils to water potential was somewhat less pronounced than the response of methanotrophic cultures (e.g., *Methylosinus trichosporium* OB3b, *Methylomonas rubra* [= *M. methanica*], an isolate from a freshwater peat, and an isolate from an intertidal marine mudflat). However, unlike soils, methanotrophic cultures exhibited a stronger adverse response to nonionic solutes than to sodium chloride.

Water regimens in soils vary substantially in space and time. Evapotranspiration increases solute concentrations and decreases matric potentials, while wetting (e.g., precipitation, snow melt, or irrigation) has the opposite effect. Changes in solute concentrations and matric potentials can shift the total soil water potential, Ψ_T, from values of >-0.1 to <-5 MPa; the higher values result in minimal physiological stresses for the soil microbiota, while the lower values pose severe limitations (see reference 8 for a review of water potential and the physiology of water stress).

Gas exchange between soils and the atmosphere and gas transport within soils vary as a function of water content (e.g., reference 10). For most populations of soil hydrogenotrophs, carboxydobacteria, and methanotrophs, gas transport from the atmosphere into the soil matrix provides the primary source of substrate (12, 34). This is especially true for subsurface populations of methanotrophs that consume atmospheric methane. A variety of evidence indicates that diffusive transport limits these bacteria (e.g., references 1, 3–5, 13, 15, 17, 18, and 21) and that atmospheric methane consumption responds sensitively to soil water content, with a distinct optimum near 20%, depending on the specific soil (10, 38). The observed optima must represent a balance between the counteracting effects of an increased methane supply rate and decreased water potentials that accompany decreasing water contents.

We examined the response of methane consumption to water stress in forest soil. The relative significance of gas transport versus water stress was assessed by comparing the responses of soils to varied water potentials with both variable and constant water contents. Results obtained with soils were compared to the responses of several different methanotrophic cultures to determine if the organisms that consume atmospheric methane have adapted to water potentials characteristic of the soil environment.

MATERIALS AND METHODS

Soil analyses. Intact soil cores were collected from a mixed hardwood-conifer forest adjacent to the Darling Marine Center, Walpole, Maine, by using acrylic tubes (6.4-cm inner diameter). Details of the site and sample collection have been published previously (21). Soils from the 4- to 8-cm depth interval were obtained by sectioning the cores and sieving them as described by Schnell and King (32). For most experimental manipulations, 10 g (fresh weight) of soil was placed into 120-ml glass jars; soil water potentials were adjusted by adding equal volumes of solutions containing various concentrations of sucrose or inorganic salts. The total liquid volume added was chosen to yield final soil water contents of 25 to 30% (grams of water [gram dry weight of soil]$^{-1}$). Deionized water was added to soils used for control treatments. Solutions were pipetted as uniformly as possible onto the soils; subsequently, the soils were mixed thoroughly but gently and allowed to equilibrate for approximately 1 h prior to analyses of methane uptake. The water contents and potentials of a separate set of soils were manipulated by air drying or wetting soils with deionized water as necessary to produce a range of water contents of 18 to 38%. Quadruplicate samples of soil with each water content were incubated with either atmospheric or 200-ppm methane in sealed jars for uptake analyses.

Methane consumption was analyzed by gas chromatography (21) after sealing the jars with butyl rubber stoppers that did not produce acetylene. Methane consumption rates for headspace concentrations of >100 ppm were calculated from linear regressions of methane depletion over time. For headspace methane concentrations of <100 ppm, uptake rate constants were estimated from linear regression of logarithmically transformed data.

Water potentials were measured in subsamples of soils treated with sucrose or salt solutions by using the dew point mode of a psychrometer (Wescor Inc., Logan, Utah) and a C-52 sample chamber. The psychrometer response was calibrated with solutions of sodium chloride; the molality of these solutions was used to calculate water potential (8). The operating parameters for the chamber were 2 min of thermal equilibration, 1 min of cooling, and 4 min of reequilibration prior to reading of the voltage output. Soil potentials were calculated from the mean of three to five replicates. Soil water content was determined gravimetrically by oven-drying the samples at 110°C for 48 h.

Culture analyses. Methanotrophs were enriched from the surface sediments of an intertidal mudflat with a high organic load, contributing to rapid sulfate depletion and moderate rates of methanogenesis (16), and from subsurface peats of a local wetland (30, 31). The enrichments used about 3 cm^3 of sediment or peat incubated in 30 ml of bicarbonate-buffered mineral medium (39) modified with sodium chloride (340 mM) and magnesium chloride (15 mM) for the marine samples. Samples were incubated with a gas phase of 5 to 10% methane and 10% carbon dioxide in air on a rotary shaker at 100 rpm and 25°C. Methane was replenished regularly; after several transfers in fresh mineral medium, the iso-

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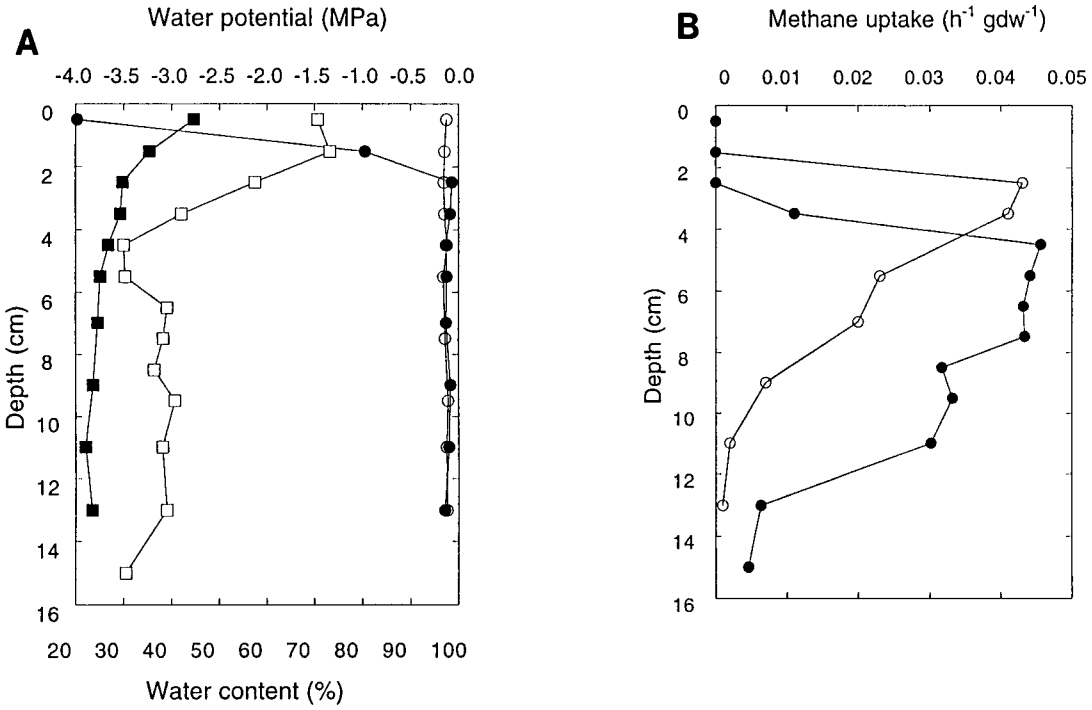


FIG. 1. (A) Depth profiles of water potential (○, ●) and water content (□, ■) for 20 September (closed symbols) and 15 November (open symbols) 1994. (B) Methane uptake rate constants (open and closed symbols for the same dates as in A). (dw, dry weight).

lates were obtained by repeated streaking of colonies on agar plates incubated under an atmosphere of 30 to 50% methane in air.

Liquid cultures of the peat and marine isolate were each grown in 100 ml of mineral medium with 50% methane in air, harvested during logarithmic growth by centrifugation (10,000 × *g*; 10 min; 4°C), and resuspended in fresh basal mineral medium without sodium chloride. The water potential of the medium was adjusted to produce a range of values by adding sodium chloride at various concentrations to cell suspensions with a final A_{600} of 0.5; water potentials were measured by using a Wescor psychrometer as described above. Methane consumption at different water potentials was monitored with 10-ml cell suspensions incubated with a headspace of 500-ppm methane in 160-ml serum bottles. All incubations were conducted in triplicate at 25°C with rotary shaking.

Cultures of *Methylosinus trichosporium* OB3b and *Methylomonas rubra* (= *M. methanica* [6]) were obtained from R. S. Hanson (University of Minnesota) and grown in nitrate-mineral salts medium (21). Cells in the late log phase of growth were harvested by centrifugation (10,000 × *g*, 4°C, 10 min), washed twice with phosphate buffer (10 mM, pH 7), and resuspended in fresh medium to an A_{600} of 0.2 to 0.3. Water potential was adjusted with sucrose or salts; the maximum concentrations used were approximately 0.8 and 0.6 M, respectively. Water potentials of the media were calculated from the final molal concentrations of all medium components. Cultures were incubated in sealed duplicate 16-ml serum bottles with 1% methane at 25°C with rotary shaking. Methane uptake was measured by analysis of headspace concentrations as described above. Subsequent to completion of an uptake time course, the cultures were subsampled for analysis of A_{600}; the concentration of bacterial biomass was estimated from an empirically derived relationship between absorbance and dry weight (30). On the basis of microscopic examination, cell size and appearance were not visibly affected by incubations in sucrose and sodium chloride solutions; thus, decreases in absorbance were attributed to cell loss.

Triplicate-washed suspensions of *M. rubra* (75 ml) were also incubated with rotary shaking in sealed 250-ml flasks with headspaces of 10% methane in air. The growth media were adjusted to a water potential of −2.2 MPa with either sucrose, sodium chloride, potassium chloride (each at about 0.6 M), or sodium chloride with 100 mM glycine betaine (GBT; final water potential, −2.4 MPa). Growth of the cultures was monitored by periodically measuring the A_{600} of medium subsamples obtained by syringe and needle.

RESULTS

Depth profiles of water potential and methane consumption. Depth profiles obtained during late summer and fall of 1994 indicated that fluctuations in water potential occurred primarily in the upper 4 cm of the soil as a consequence of periodic drying and precipitation. On 20 September 1994, subsequent to a relatively dry period, the water potential in surface soils was −4 MPa and increased to −0.2 MPa at a depth of about 8 cm (Fig. 1A). However, relatively uniform profiles with moderate potentials (Ψ_τ = −0.12 to −0.16) were obtained on 15 November 1994 (Fig. 1B) after a period of increased precipitation. Irrespective of water potential variations, methane consumption in surface soils was consistently low and increased with depth to a subsurface maximum (Fig. 1A and B); the magnitude of the subsurface methane consumption maximum was relatively stable temporally.

Interactions among methane consumption, soil water potential, and soil water content. Water potential in a set of soils air dried or wetted to produce water contents of 18 to 38% varied from about −0.03 to −0.97 MPa. Rate constants for atmospheric methane consumption in these soils were greatest (about 0.058 h^{-1} [g dry weight of soil]$^{-1}$) at a water potential and content of −0.22 MPa and 25.5%, respectively (Fig. 2A); higher and lower water contents and potentials resulted in decreased activity. The relative changes in atmospheric methane consumption were roughly symmetrical about the maximum. Soils air dried to water contents as low as 3% retained limited methanotrophic activity; however, when soils were dried to water contents of <15% and then wetted to 33%, only a fraction of the control activity was recovered during incubations of <1 day (20).

For a parallel set of soils incubated with 200-ppm methane, maximal activity (2.6 nmol h^{-1} [g dry weight of soil]$^{-1}$) was observed at the highest water contents and potentials (about 36 to 38% and −0.01 to −0.03 MPa; Fig. 2A). Methane uptake by these soils tended to decrease monotonically with decreasing water content and potential. Uptake at a potential of about −1 MPa was <13% of the maximum uptake rate; in contrast,

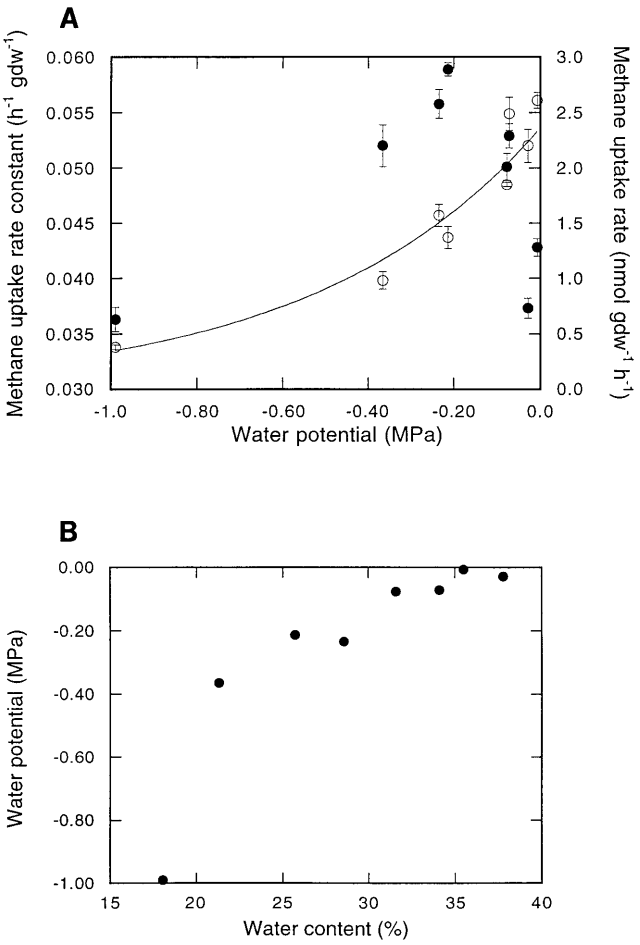


FIG. 2. (A) Methane uptake as a function of water potential in soils air dried to various water contents. Symbols: ●, soils incubated with atmospheric methane; ○, soils incubated with 200-ppm methane. (B) Relationship between soil water potential and soil water content. Error bars represent ± 1 standard error of the mean (n = 4). dw, dry weight.

atmospheric methane consumption at −1 MPa was about 62% of the maximum.

Response of methane consumption to ionic and nonionic solutes. Soil water potential was varied by adding either sucrose or an inorganic salt (sodium chloride, sodium sulfate, or potassium chloride) to soils with a constant water content. Under these conditions, methane consumption decreased with decreasing soil water potential (Fig. 3). With the exception of an initially abrupt decrease in uptake of 200-ppm methane, the relationships between uptake and potential were similar for atmospheric and superatmospheric methane concentrations. However, methane uptake was substantially lower in soils to which salts rather than sucrose had been added to adjust the water potential (Fig. 3). For example, the total decrease in activity over a water potential range of −0.01 to −1.0 MPa was about 27.5% for soils treated with sucrose; in contrast, activity decreased by 75 to 93%, for soils treated with KCl or NaCl, respectively. Greater decreases in methanotrophic activity at a given water potential were observed for sodium and potassium chlorides than sodium sulfate at atmospheric and superatmospheric methane concentrations (19, 20).

Effect of water potential on methanotrophic activity by pure cultures and isolates. Freshwater and marine isolates responded similarly to variations in water potential based on

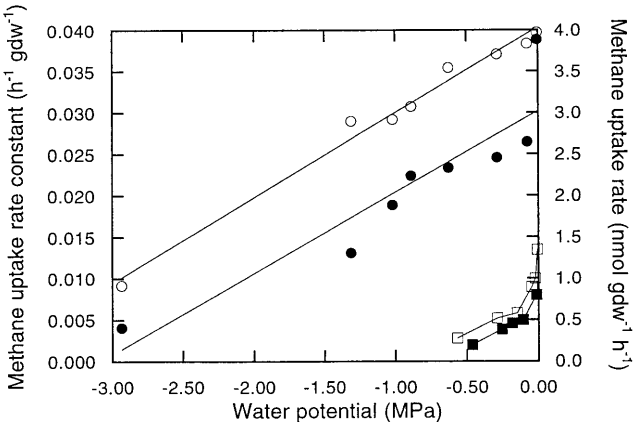


FIG. 3. Methane uptake rate constants in soils incubated with atmospheric methane at a constant water content with water potential varied by addition of sucrose, NaCl, or KCl (○, □, ■; left scale) or with 200-ppm methane and sucrose (●; right scale). Error bars represent ± 1 standard error of the mean (n = 3). dw, dry weight.

additions of sodium chloride to a basal medium (Fig. 4). Methane consumption decreased by over 97 and 84% for the two respective cultures with a decrease in potential to −3.1 MPa. Although the marine isolate was obtained from a site exposed to full seawater salinities, it showed no adaptation or preference for elevated salinity or depressed water potential. In addition, methane uptake per unit of biomass was considerably lower for the marine isolate than for the freshwater isolate. Comparisons of uptake per unit of biomass between the isolates and pure cultures are not readily interpreted because of the large difference between the methane concentrations used for the incubations and the lack of data on the kinetic responses of the various organisms.

Methane consumption per unit of biomass by *M. trichosporium* OB3b also decreased approximately exponentially with decreasing water potential (Fig. 5A). Relative to the basal medium (Ψ = −0.15 MPa), activity was reduced by 88% in a medium with a potential of −2.8 MPa. Although the activities per unit of biomass in media with either sucrose or sodium chloride were essentially equivalent, the biomass in media with

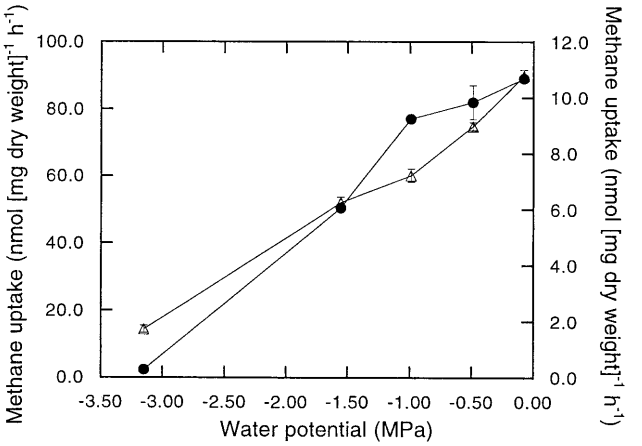
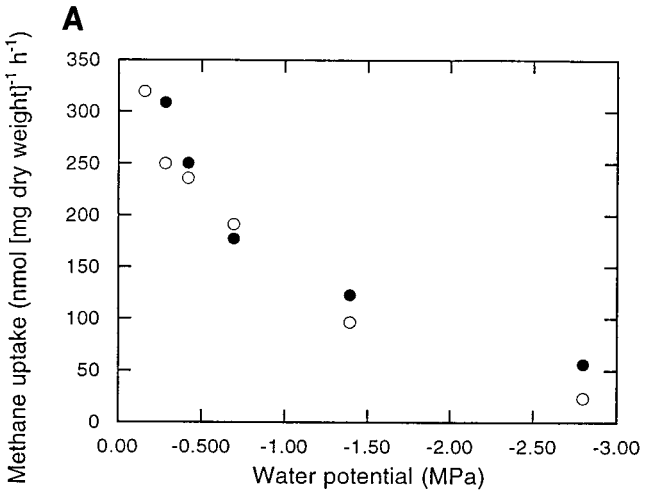


FIG. 4. Methane uptake by an isolate from a freshwater peat (△; left scale) or a marine sediment (●; right scale) as a function of water potential in media adjusted with sodium chloride. Note that uptake rates were measured with a headspace methane concentration of 500 ppm. Error bars represent ± 1 standard error of the mean (n = 3).



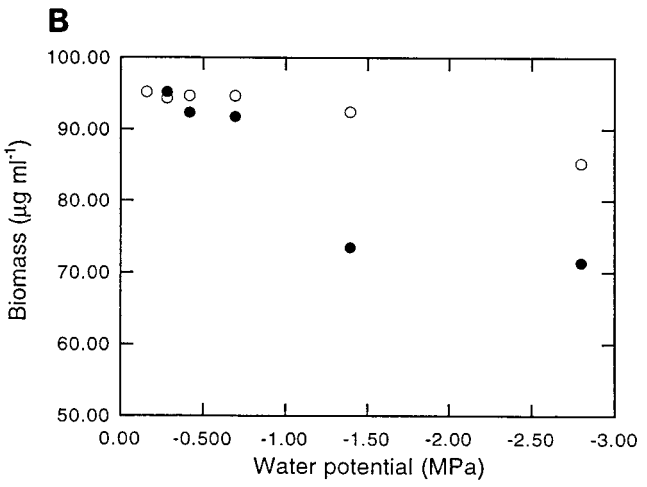


FIG. 5. (A) Methane uptake by cultures of *M. trichosporium* OB3b as a function of water potential in media adjusted with sodium chloride (○) or sucrose (●). (B) Biomass of *M. trichosporium* OB3b in media adjusted with sodium chloride (○) or sucrose (●). Note that uptake rates were measured with a headspace methane concentration of 1%.

sucrose decreased as a function of decreasing water potential while biomass remained constant in media with sodium chloride (Fig. 5B). In sucrose-treated soils, the total loss of activity (about 80%; Fig. 3) from about −0.1 to −2.9 MPa was comparable to that observed for *M. trichosporium* OB3b. However, soil methanotrophic activity was reduced to a greater extent when sodium chloride rather than sucrose was used to adjust the water potential (Fig. 3) while the reverse was observed for the loss of *M. trichosporium* OB3b biomass (Fig. 5B).

M. rubra (= *M. methanica* [6]) was also sensitive to water stress (Fig. 6). *M. rubra* grew in basal medium with sodium chloride or sodium chloride plus GBT (Ψ = −2.2 and −2.4 MPa, respectively), although in both cases growth was depressed relative to that in basal medium only; in addition, growth was unaffected by the addition of GBT. *M. rubra* was unable to grow in basal medium with potassium chloride or sucrose as the primary solute (Ψ = −2.2 MPa; Fig. 6).

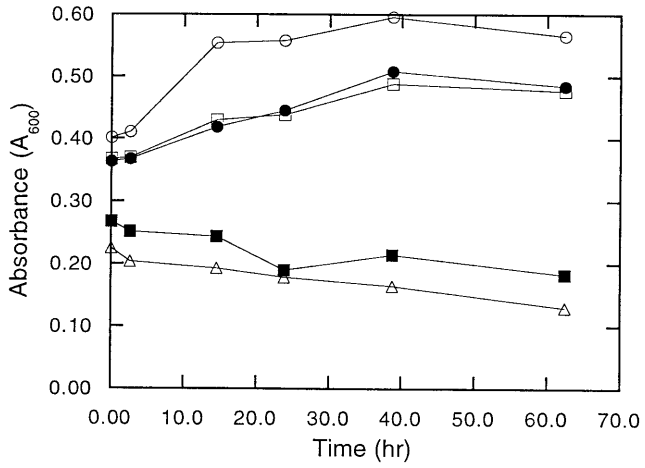


FIG. 6. Time course of *M. rubra* growth in a basal medium (○) or media with a water potential of −2.2 MPa adjusted with sodium chloride (●), sodium chloride plus 100 mM GBT (□; Ψ = −2.4 MPa), potassium chloride (■), or sucrose (△).

DISCUSSION

Gas transport through soil has been proposed as a key rate-limiting factor for atmospheric methane consumption (e.g., references 1, 4, 5, 10, 13–15, 21, and 24). For example, Whalen et al. (38) attributed increased methanotrophic activity in landfill soils with lower water contents to higher rates of gas diffusion and an inverse relationship between water content and the volume of gas-filled pore spaces. Reduced methanotrophic activity at a water content of 5% may have been due to cell desiccation, although this point was not addressed by Whalen et al. (38) specifically. The relationship between water content and methanotrophic activity has prompted Whalen et al. (38) and others to propose that the significance of soils as a negative feedback for atmospheric methane and global warming might increase in the future because of decreased soil water content in a warmer, drier climate. However, decreased soil water content might also result in positive feedback if the soil methane sink is reduced because of water stress.

The relationships among methane uptake, water content, and water potential provide insights into the interactions between gas transport and water stress and a basis for predicting responses to changes in soil water regimens. A primary constraint is that soil methanotrophs are not particularly xerotolerant. Methane uptake is very strongly depressed by water potentials of −3 to −4 MPa (Fig. 2 to 5). In contrast, many gram-negative, heterotrophic eubacteria tolerate water potentials as low as −7 MPa, and fungi can tolerate potentials as low as −80 MPa (8). Comparable responses of soils (Fig. 3) and several methanotrophic isolates (Fig. 4 and 5) to decreased water potential at a constant water content may also indicate that soil methanotrophs have only a limited ability to adapt to low water potentials.

Although other heterotrophs show greater xerotolerance than methanotrophs, the response of soil nitrification is strikingly similar to that for methane consumption. Stark and Firestone (36) have shown that nitrification rates in oak-grassland soils were inhibited by >85% at water potentials of <−3 MPa. The similarity in response of nitrification and methane consumption to water stress is consistent with the high degree of overlap for other characteristics among the methanotrophs and ammonia-oxidizing bacteria (e.g., reference 2).

Water stress tolerance by soil methanotrophs does not ap-

pear to be constrained by substrate availability, as similar responses have been obtained for soils incubated with atmospheric or 200-ppm methane (Fig. 3). Earlier studies have shown that the relative extent of methane respiration is equivalent for concentrations of 1.7 and 170 ppm and that methane concentrations of <170 ppm do not stimulate growth during short-term incubations (33). Consequently, incubations with 200-ppm methane should have increased water stress tolerance if the energetic costs of osmoregulation were an important limiting factor. The energetic costs of osmoregulation could be significant, but apparently other factors play a greater role.

The response of methane consumption in soils where water content and potential covaried (Fig. 2A and B) illustrates the relative importance of gas transport and water stress. Limitation primarily by gas transport is consistent with the increases in atmospheric methane uptake accompanying decreases in water content of 38 to 25.5%, a range over which water potential decreased from about −0.03 to −0.23 MPa. Water stress limitation explains the 39% reduction in atmospheric methane uptake with reductions in water content of 25.5 to 18% and water potential to −0.97 MPa (Fig. 2A).

In contrast, limitation by water stress appears to account entirely for the pattern of activity in soils incubated with 200-ppm methane, as uptake decreased continuously with decreasing water content and potential (Fig. 2B). Alleviating transport limitation by incubating soils with elevated methane concentrations resulted in manifestation of water stress at potentials of <−0.01 MPa, in accord with results obtained with the methanotrophic isolates (Fig. 4 and 5). The 58% increase in atmospheric methane uptake and the 37% decrease in the uptake of 200-ppm methane over the water content range of 38 to 25.5% provide an indication of the relative significance of gas transport limitation at atmospheric methane levels.

Stark and Firestone (36) have also described a shift in the relative importance for nitrification of solute transport and water stress as a function of water potential. Decreases in water content and potential result in more tortuous diffusion paths for solutes and thus decrease rates of diffusive flux to cells. For nitrification, this effect appears most important for potentials of >−0.6 MPa; at potentials of <−0.6 MPa, the effects of water stress are most important (36). Data presented here indicate that water stress becomes the most important limiting factor for atmospheric methane uptake between −0.2 and −1.0 MPa, in agreement with the results of Stark and Firestone (36).

Parallels between the responses of methanotrophic isolates and soils indicate that the behavior of pure cultures might be used to understand the physiological ecology of water stress. The similarity in the responses of the four isolates in this study (Fig. 4 and 5), which originated from marine sediments, soil, and freshwater peats and muds, supports this contention. The absence of a unique water stress response by the marine isolate (Fig. 4) suggests that methanotrophs as a group may be relatively water stress intolerant. This intolerance may explain partially the limited success reported for isolating or detecting methanotrophs in hypersaline environments (11, 35).

The water stress response in methanotrophs undoubtedly involves methane monooxygenases, as well as other enzyme systems, and appears to vary depending on the nature of the stress. For example, *M. rubra* could grow in a medium adjusted to −2.1 MPa with sodium chloride but not with potassium chloride (Fig. 6). This may reflect an atypical sensitivity to potassium, since potassium ions are generally less destabilizing as a solute than are sodium ions (8). In soils, the responses to sodium and potassium chlorides are equivalent. However, it is not clear whether soil methanotrophs are less sensitive to potassium ions than are cultures. The response of soils to additions of any of the alkaline metal or alkaline earth ions is complicated by desorption of ammonium from clays (19). Ammonium inhibits methane oxidation in soils (e.g., 9, 22, 23, 27, 32, 37), confounding comparisons with cultures that do not contain ammonium. Regardless, the results for *M. rubra* suggest that differences in ionic composition of the soil solution could contribute to variable responses to water potential among soils.

The lack of response by *M. rubra* to GBT is also noteworthy, since exogenous GBT at concentrations lower than that used in this study ameliorates salt stress in a variety of eubacteria, including taxa that are incapable of producing it endogenously (e.g., 25, 26, 29). A number of questions about the physiology of water stress in methanotrophs are unresolved. Can methanotrophs transport GBT or other exogenous compatible solutes? Do methylated compatible solutes (e.g., GBT and dimethylsulfoniopropionate) adversely affect methane monooxygenases? What compatible solutes are accumulated in response to water stress? Resolution of these questions is important for understanding the constraints on the ability of methanotrophs to adapt to low water potentials in situ.

Additional questions arise from the behavior of *M. rubra* and *M. trichosporium* OB3b in solutions with water potentials adjusted with sodium chloride, a permeable solute, and sucrose, an impermeable solute. Although rates of methane oxidation per unit of biomass were comparable for sodium chloride and sucrose, the biomasses of both methanotrophs decreased substantially in solutions of the impermeable solutes (Fig. 5 and 6).

These results can be explained by considering the responses of cells upon transfer from high to low water potential media. Since positive turgor pressure is required for bacterial growth, actively growing cells that have equilibrated with a given medium must have a lower internal water potential than the potential of the medium according to $\Psi_e = \psi_i + \psi_p$, where Ψ_e is the total external water potential, ψ_i is the internal water potential (matric plus osmotic), and ψ_p is the turgor pressure potential (8). On transfer to a medium with a lower potential than that to which the cells have equilibrated, loss of intracellular water results in decreased turgor and ψ_i; when permeable (or transportable) solutes are present in the medium, water losses can be partially offset by the volume flow of solutes into cells. Subsequent to the initial equilibration (lasting seconds [7]), cells must restore turgor and adjust intracellular solute concentrations prior to active growth; this process may include the uptake or synthesis of compatible solutes (8).

When impermeable solutes primarily determine water potential in a medium, restoring an adequate internal water potential and solute composition becomes a severe challenge. The magnitude of the challenge is illustrated by estimating the change in molality of an intracellular solution if the permeable ions are accumulated from a medium dominated by impermeable solutes: assuming a cell water volume of 7.85×10^{-13} cm^3 cell^{-1}, a water density of 1.0 g cm^{-3}, and conditions comparable to those in this study (cell density of 10^9 cm^{-3} of medium and 50 μmol of ideal permeable ions cm^{-3} of medium), the final ion concentration in the cells of a hypothetical bacterial population would be about 0.3 molal above the initial concentration, resulting in a maximum $\Delta\psi_i$ of about −0.8 MPa at 25°C. This might be sufficient to support some level of adaptation at moderate levels of water stress (e.g., −1 MPa) but would clearly be insufficient for more substantial stresses.

Relative to media containing sodium chloride, the loss of *M. trichosporium* OB3b and *M. rubra* biomass at all levels of sucrose tested (Fig. 5 and 6) strongly suggests that the availability

of permeable salts determines, in part, the response of methanotrophs to water stress. However, the equivalence of biomass-specific methane oxidation rates in media with sucrose or sodium chloride (Fig. 5) suggests that those cells that survive or adapt to water stress do so similarly well, irrespective of solute permeability; in addition, the marked inhibition of *M. rubra* growth in media containing KCl rather than NaCl indicates that the specific composition of the ionic medium is a critical determinant of adaptation to water stress. Although not specifically explored here, the availability of methane must also play a role in water stress responses, since the accumulation and synthesis of compatible solutes and import and export of other solutes require energy. Methane supply may be a particularly important factor in soils, as indicated by the response of atmospheric methane uptake to variable water potential and content (Fig. 3).

The stronger inhibition of methane consumption in soils by sodium and potassium chlorides than sucrose (Fig. 2) appears to contradict the model described above for cultures. However, ammonium desorption by salts with attendant inhibition of methane consumption could account for the discrepancy (19). Thus, methanotrophs in soils treated with sucrose would have to respond primarily to water stress, while those in soils treated with salts would have to deal with the additional effects of ammonium toxicity. This hypothesis might be tested by using various additions of sucrose, sucrose plus ammonium, salts, and salts plus ammonium.

A more difficult hypothesis to test concerns the role of water potential as a factor limiting the distribution of methanotrophs within the soil profile. A number of studies have shown that atmospheric methane consumption is localized in the mineral horizon, well below the soil surface, where methane concentrations are highest. Although the gas-filled pore space may be higher at a given water potential in surface soils than in the mineral horizon and surface soils can have relatively high water potentials (Fig. 1), the evaporative drying of surface soils may periodically result in potentials that preclude growth or colonization by methanotrophs. The potential significance of periodic drying is illustrated by results of Nesbit and Breitenbeck (28), who showed that the methanotrophic activity of air-dried soils was not recovered after rewetting. The effect of intermittent drying may be exacerbated by deposition of ammonium on and rapid rates of ammonification in surface soils.

Although some have predicted that enhanced atmospheric methane consumption might accompany somewhat drier conditions in the future because of an increase in the gas-filled pore space, the potential for increased uptake rates might be offset by localization of methanotrophic activity even deeper in the soil horizon than at present. Drier conditions would likely increase the depth of the soil profile exposed to large shifts in water potential, including potentials that adversely affect methanotrophic activity. Shifts in the downcore regimens of water content and potential would undoubtedly affect other parameters, such as fungal distribution and activity, that might adversely affect methane uptake as well. Clearly, reliable predictions of the response of atmospheric methane consumption in soils to climate change require more extensive information on the physiological ecology of soil methanotrophs.

ACKNOWLEDGMENT

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