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## Response of Atmospheric Methane Consumption by Maine Forest Soils to Exogenous Aluminum Salts†

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**Atmospheric methane consumption by Maine forest soils was inhibited by additions of environmentally relevant levels of aluminum. Aluminum chloride was more inhibitory than nitrate or sulfate salts, but its effect was comparable to that of a chelated form of aluminum. Inhibition could be explained in part by the lower soil pH values which resulted from aluminum addition. However, significantly greater inhibition by aluminum than by mineral acids at equivalent soil pH values indicated that inhibition also resulted from direct effects of aluminum per se. The extent of inhibition by exogenous aluminum increased with increasing methane concentration for soils incubated in vitro. At methane concentrations of >10 ppm, inhibition could be observed when aluminum chloride was added at concentrations as low as 10 nmol g (fresh weight) of soil<sup>-1</sup>. These results suggest that widespread acidification of soils and aluminum mobilization due to acid precipitation may exacerbate inhibition of atmospheric methane consumption due to changes in other parameters and increase the contribution of methane to global warming.**

A number of factors adversely affect atmospheric methane consumption by soils. Ammonium is one of the most important of these (6, 8, 13, 14, 20, 30, 31, 37, 49), but other factors include water stress (47), terpenes (3), salts (1, 21, 27, 32), and land use (22, 23, 25, 28). Soil pH has also been documented as a potentially important limiting factor, with both acidic (pH <4) and alkaline (pH >7) regimes inhibiting activity (2, 12, 22; J. Benstead and G. M. King, unpublished results). Although some evidence supports a role in methane consumption for acid-tolerant or moderately acidophilic methanotrophs in peats (12), the acid-tolerant peat isolates described to date have not been shown to consume atmospheric methane, and acid-tolerant methanotrophs have not been documented for soils.

In contrast to the impact of pH in peats, the effects of pH on methanotrophic activity in acidic soils may be compounded by solubilization of aluminosilicates, which constitute a major fraction of the mineral horizons where atmospheric methane consumption occurs most actively. Although the chemistry of aluminum is well understood (36) and its toxic effects on multicellular organisms are known in some detail (15, 17, 34–36, 52), the response of microbes to aluminum is not well documented (42).

Several studies have examined the effects of aluminum on cyanobacteria and fungi and documented a range of responses (10, 41–43). Physiological studies with bacteria have emphasized well-known strains, such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus megaterium*, and *Pseudomonas fluorescens* (4, 11, 19, 42, 44). A broader range of studies have focused on rhizobia and documented toxicity in cultures and in bacterium-legume symbioses (7, 8, 18, 24, 26, 33, 38, 39, 43, 50, 51). However, the effects of aluminum on microbes or microbial processes in a more general ecological context have not been adequately assessed.

Nonetheless, dissolved aluminum concentrations can reach toxic levels in soil solutions with pH values of <4.8 or >7.4. These values occur naturally in many soils and are increasingly common because of widespread acidification associated with acid precipitation (42). Acidification clearly mobilizes aluminum and has been associated with significant impacts on plant and animal populations (7, 16, 48). Whether microbial processes in soils are similarly affected is not certain.

We report here responses of atmospheric methane consumption in Maine forest soils to exogenous aluminum salts. At aluminum concentrations of >1  $\mu\text{mol g}$  (fresh weight) (gfw) of soil<sup>-1</sup>, atmospheric methane consumption decreased as a consequence of direct effects of aluminum and indirect effects due to decreased soil pH. The effects of aluminum were comparable for several different forms (a citrate chelate and nitrate and sulfate salts) but were greatest for the chloride salt. At concentrations of <1  $\mu\text{mol gfw}$  of soil<sup>-1</sup>, which did not affect soil pH significantly, aluminum had a dramatic effect on the kinetics of methane consumption: the maximum uptake velocity ( $V_{\text{max}}$ ) decreased markedly and there were somewhat smaller and more variable decreases in the apparent  $K_m$ . In contrast, addition of ammonium decreased the  $V_{\text{max}}$  and increased the apparent  $K_m$ .

### MATERIALS AND METHODS

The effects of aluminum salts on methane consumption were determined by using sieved (2-mm mesh) A-horizon soils from the depth of greatest methanotrophic activity, 6 to 10 cm, in a mixed conifer-hardwood forest at the Darling Marine Center. Various characteristics of the site have been described previously (1, 29–33, 46). For routine assays, 10-gfw soil samples were transferred to glass jars with a headspace of about 110 cm<sup>3</sup>. One-half-milliliter volumes of deionized water, stock solutions containing an aluminum salt (chloride, nitrate, or sulfate), or sulfuric acid were pipetted carefully onto the soil samples, each of which was mixed gently but thoroughly with a small spatula. The final concentrations of added aluminum ranged from <0.1 to 8  $\mu\text{mol of Al gfw of soil}^{-1}$ . Sulfuric acid or other mineral acids were added at concentrations based on the maximum proton production expected from aluminum hydrolysis (i.e., a ratio of 3 H<sup>+</sup> to 1 Al<sup>3+</sup>). After aluminum stocks, acid, or deionized water was added, the jars were sealed with butyl rubber stoppers that did not release detectable levels of methane or other hydrocarbons. The initial methane concentrations in the jar headspaces ranged from the atmospheric concentration to 1%, with superatmospheric levels obtained by adding ultra-high-purity methane as needed. For these and all

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other assays, the soil water contents were 25 to 35% as determined by drying soils for 24 h at 105°C.

Methane uptake rates were determined by using time course measurements of headspace subsamples (0.3 cm<sup>3</sup>) removed from the jars with a needle and syringe for assay by flame ionization gas chromatography with a Shimadzu GC-14AM gas chromatograph as described by King and Adamsen (29). Detector responses were analyzed with an HP-3396 integrator (Hewlett-Packard, Inc.) and standardized with 3.16 ppm of methane in nitrogen (Maine OxyAcetylene, Inc.). For initial headspace methane concentrations of <10 ppm, methane uptake rate constants were estimated from a nonlinear regression analysis (Kaledigraph; Adelbeck Software, Inc.) of exponential decreases over time; linear regressions were used for initial headspace concentrations of >10 ppm. All treatments were run in triplicate.

The effects of chelated aluminum compared with those of nonchelated aluminum were assessed by preparing stock solutions of aluminum chloride with sodium citrate, with concentration of the latter one-third of the aluminum concentration in accord with the stoichiometry of aluminum citrate complexes. Treatments consisted of adding aluminum chloride, aluminum citrate, or citrate to soil at final concentrations of either 0.1 or 1.6  $\mu\text{mol gfw of soil}^{-1}$ . Initial headspace methane concentrations of 1.8 and 64 ppm were used for each of the salt treatments. Uptake rates were assessed as described above in triplicate.

Kinetic parameters for methane uptake were determined after addition of 0, 0.01, 0.04, 0.1, 0.4, or 1.6  $\mu\text{mol of Al}^{3+}$  (as the sulfate salt) gfw of soil<sup>-1</sup>. At each of these Al<sup>3+</sup> concentrations, soils were incubated with headspace methane concentrations of 1.8, 8, 16, 32, 64, 120, and 240 ppm. Methane uptake rates were determined as before. Apparent half-saturation constants ( $K_s$ ) and  $V_{\text{max}}$  were estimated from nonlinear regression analysis by using a Michaelis-Menten model and Kaleidagraph software (Adelbeck Software). Similar assays were conducted by using ammonium chloride at final concentrations of 0 to 4  $\mu\text{mol gfw of soil}^{-1}$  and methane headspace concentrations of 1.8, 5, 10, 20, 50, and 100 ppm.

The ability of added aluminum to desorb ammonium was measured by adding 0.5-ml volumes of aluminum sulfate or sulfuric acid stock solutions to triplicate 10-gfw soil samples, producing final added concentrations of 1  $\mu\text{mol of Al}^{3+}$  or 3  $\mu\text{mol of H}^+$  gfw soil<sup>-1</sup>. Ammonium was subsequently extracted by adding deionized water and centrifuging the slurries (32). Ammonium concentrations were assayed by a salicylate-hypochlorite colorimetric method described by Bower and Holm-Hansen (5).

## RESULTS

Atmospheric methane uptake rate constants decreased with increasing aluminum sulfate concentrations (Fig. 1). Compared to deionized water controls, the uptake rates were reduced by approximately 50 and 90% for additions of 1 and 8  $\mu\text{mol of Al gfw of soil}^{-1}$ , respectively. The uptake rate constants also decreased as a function of increasing sulfuric acid concentrations (Fig. 1A). The decreases in the uptake rate constants for the aluminum and sulfuric acid treatments were fit using nonlinear regression to a relationship of the following form (Fig. 1A):  $RC = RC_{\text{final}} + (RC_{\text{init}} - RC_{\text{final}})e^{-kX}$ , where RC is the uptake rate constant,  $RC_{\text{init}}$  is the initial uptake rate constant value,  $RC_{\text{final}}$  is the final uptake rate constant value,  $k$  is a decay or inhibition constant, and  $X$  is the concentration of Al or H<sup>+</sup> added to the soils. For this relationship, the inhibition constants for Al and H<sup>+</sup> were  $0.699 \pm 0.066$  and  $0.177 \pm 0.027$ , respectively.

Since the hydrolysis of  $\text{Al}(\text{H}_2\text{O})_6^{3+}$  is accompanied by up to 3 H<sup>+</sup> equivalents [i.e.,  $\text{Al}(\text{H}_2\text{O})_6^{3+} \rightarrow \text{Al}(\text{H}_2\text{O})_3 + 3\text{H}^+$ ], the estimates indicated that acidification itself might have accounted for about 76.1% of the observed inhibition, with other direct effects of Al accounting for the remainder (23.9%). However, the soil pH was lower (Fig. 1B) for sulfuric acid treatments than for aluminum treatments at comparable levels of proton addition (i.e., at a ratio of 3 H<sup>+</sup> equivalents for sulfuric acid per mol of aluminum sulfate). Furthermore, the methane uptake values for soils at comparable pH values were lower for aluminum sulfate treatments than for sulfuric acid treatments (Fig. 1C). Thus, the level of inhibition directly attributable to aluminum was likely greater than that indicated by the preceding calculation.

Aluminum chloride was significantly more inhibitory than the nitrate and sulfate salts at a concentration of 1  $\mu\text{mol of Al gfw of soil}^{-1}$  for a methane concentration of 100 ppm; inhibition by aluminum nitrate and inhibition by aluminum sulfate

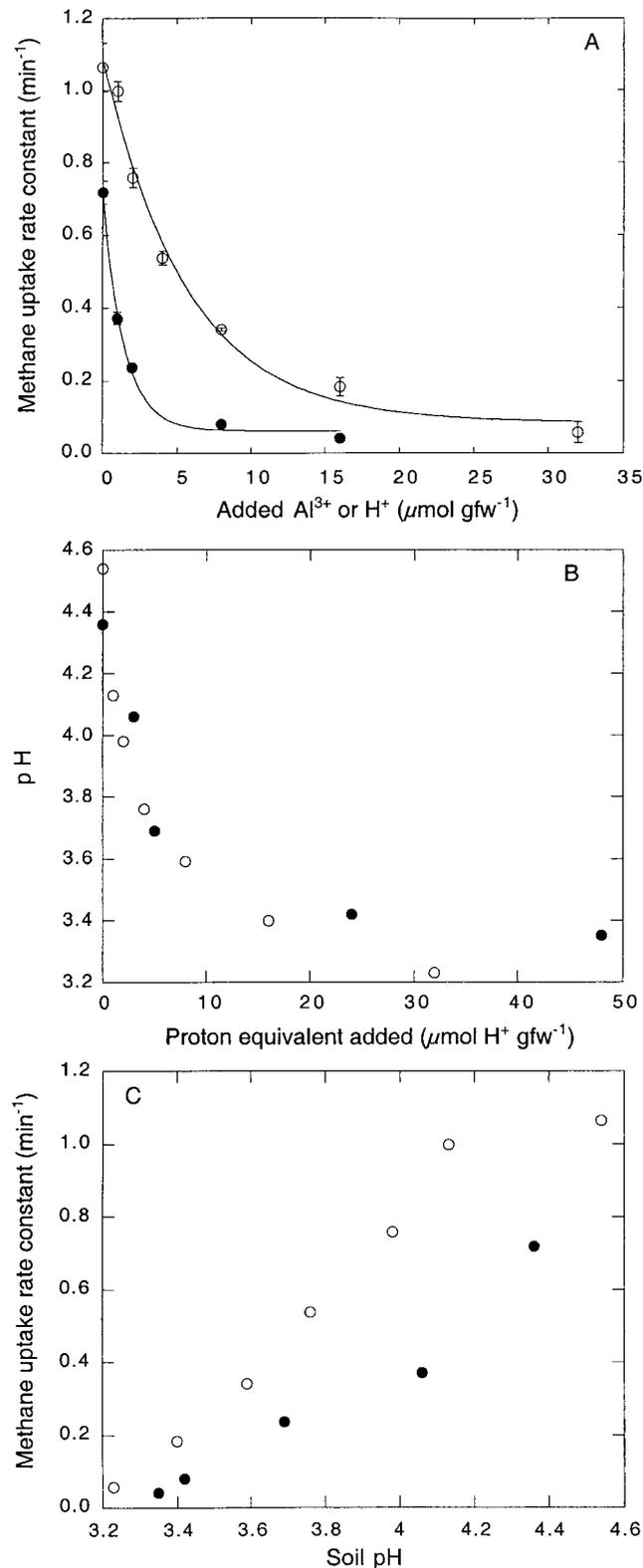


FIG. 1. (A) Atmospheric methane uptake rate constants for soils amended with various concentrations of aluminum sulfate (●) or sulfuric acid (○). Data are means of triplicate determinations  $\pm 1$  standard error. (B) Plot of soil pH versus amount of proton equivalent added for aluminum sulfate (●) or sulfuric acid (○). Note that the molar ratio of proton equivalents is 3:1 for aluminum additions. (C) Plot of methane uptake rate constants for aluminum sulfate (●) or sulfuric acid (○) versus soil pH.

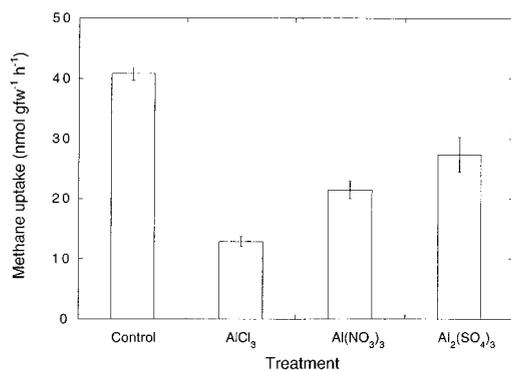


FIG. 2. Methane uptake rates for various aluminum salt additions ( $1 \mu\text{mol of Al}^{3+} \text{ gfw}^{-1}$ ). Soils were incubated with an initial headspace methane concentration of 100 ppm. Data are means of triplicate determinations  $\pm 1$  standard error.

did not differ statistically (Fig. 2). For each of the salts, the extent of inhibition was greater at 100 ppm of methane than at atmospheric methane concentrations (data not shown). The trend of increasing inhibition with increasing methane concentrations was confirmed by incubating soils containing aluminum chloride at  $2 \mu\text{mol gfw of soil}^{-1}$  with headspace methane levels ranging from the atmospheric concentration to 10,000 ppm (Fig. 3). Compared to controls without aluminum, aluminum inhibition was greatest for 1,000 ppm of methane, and lower levels of inhibition occurred at methane concentrations below or above this level.

Addition of aluminum in a chelated form (as a citrate complex) did not affect the inhibition patterns. Neither aluminum chloride nor aluminum citrate at  $0.1 \mu\text{mol of Al gfw of soil}^{-1}$  significantly decreased atmospheric methane consumption compared to a treatment with citrate alone or deionized water controls. However, both aluminum treatments, but not the citrate treatment, decreased methane uptake at headspace concentrations of 64 ppm (Fig. 4A). At concentrations of  $1.6 \mu\text{mol of Al gfw of soil}^{-1}$ , the effects of the chloride salt and the citrate complex were also comparable, but in this case inhibition was observed for both atmospheric methane and 64 ppm of methane, with greater inhibition for the latter (Fig. 4B).

A kinetic analysis revealed consistently low apparent  $K_m$

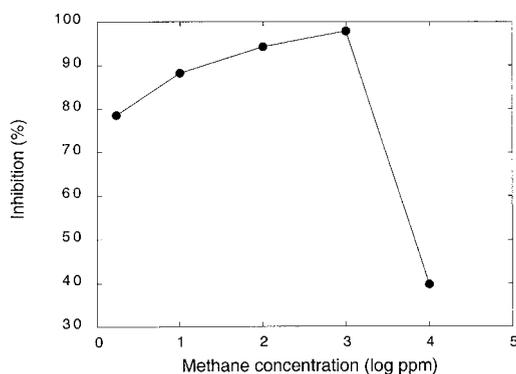


FIG. 3. Percent inhibition of methane consumption by  $\text{AlCl}_3$  ( $2 \mu\text{mol of Al}^{3+} \text{ gfw}^{-1}$ ) for soils incubated with various methane concentrations (atmospheric concentration to 10,000 ppm). Percent inhibition was determined from the ratio of methane uptake for aluminum-treated soils to methane uptake for untreated soils at each methane concentration. Data are means of triplicate determinations  $\pm 1$  standard error.

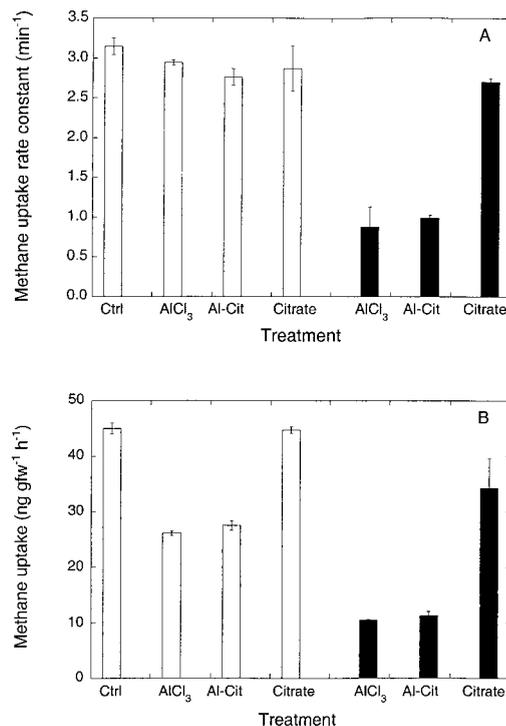


FIG. 4. (A) Methane uptake rate constants for soils incubated with atmospheric methane after addition of  $\text{AlCl}_3$ , aluminum citrate, or citrate at a concentration of  $0.1 \mu\text{mol of Al}^{3+} \text{ gfw}^{-1}$  (open bars) or  $1.6 \mu\text{mol of Al}^{3+} \text{ gfw}^{-1}$  (solid bars). Note that the citrate concentrations are one-third those of aluminum. Data are means of triplicate determinations  $\pm 1$  standard error. (B) Same as panel A, but soils were incubated with an initial headspace methane concentration of 64 ppm. Ctrl, control; Al-Cit, aluminum citrate.

values ( $14.0 \pm 0.5 \text{ ppm}$ ) for soils amended with only deionized water.  $V_{\text{max}}$  values for these soils were  $53.8 \pm 12.0 \text{ ng of CH}_4 \text{ gfw of soil}^{-1} \text{ h}^{-1}$ . Addition of aluminum chloride at concentrations ranging from 0.01 to  $1.6 \mu\text{mol of Al gfw of soil}^{-1}$  tended to decrease the apparent  $K_m$ , especially at the higher aluminum concentrations, although the changes were not statistically significant ( $P > 0.1$ ) (Fig. 5). In contrast,  $V_{\text{max}}$  was

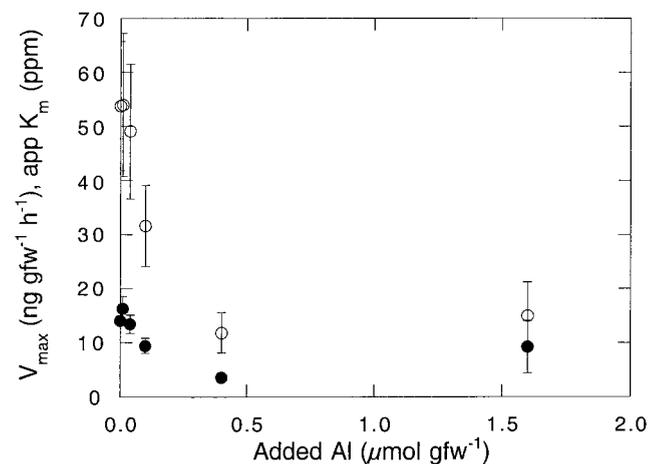


FIG. 5.  $V_{\text{max}}$  ( $\circ$ ) and apparent  $K_m$  (app  $K_m$ ) ( $\bullet$ ) as a function of added aluminum chloride. Data are means of triplicate determinations  $\pm 1$  standard error.

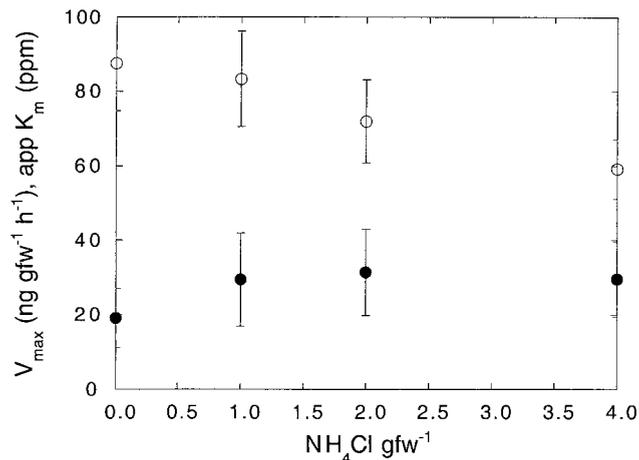


FIG. 6.  $V_{\max}$  (○) and apparent  $K_m$  (app  $K_m$ ) (●) as a function of added  $\text{NH}_4\text{Cl}$ . Data are means of triplicate determinations  $\pm 1$  standard error.

strongly depressed, with distinct inhibition apparent at 0.1  $\mu\text{mol}$  Al of gfw of soil<sup>-1</sup> (Fig. 5). Added ammonium at relatively high concentrations decreased  $V_{\max}$ , but little effect on  $V_{\max}$  was noted with ammonium at final concentrations of 0.1 to 1  $\mu\text{mol}$  gfw of soil<sup>-1</sup> (Fig. 6). Overall, the  $V_{\max}$  appeared to be more sensitive to exogenous aluminum than to ammonium. In contrast, ammonium at all concentrations increased the apparent  $K_m$  by comparable amounts (Fig. 6).

Aluminum salt or acid additions increased the ammonium concentrations in aqueous soil extracts approximately twofold compared to soils treated with only deionized water (Fig. 7). However, there were no significant differences among the aluminum or acid treatments ( $P > 0.1$ ). The amounts of ammonium mobilized by aluminum or acid additions, about 25 to 35 nmol gfw of soil<sup>-1</sup>, represented  $<1\%$  of the cation equivalents added to the soil but accounted for a large fraction (50 to 70%) of the ammonium typically found in 1 N KCl extracts of the soils.

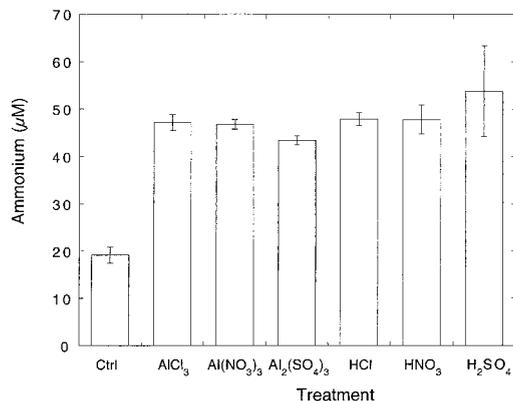


FIG. 7. Ammonium concentrations in aqueous extracts (1 ml of deionized water gfw<sup>-1</sup>) for soils incubated with 1  $\mu\text{mol}$  of aluminum salts gfw<sup>-1</sup> or 3  $\mu\text{mol}$  of acids gfw<sup>-1</sup>. Data are means of triplicate determinations  $\pm 1$  standard error. Ctrl, control.

## DISCUSSION

Exogenous aluminum inhibits atmospheric methane consumption by Maine forest soils (Fig. 1). At pH values typical of the soils used in this study ( $\text{pH} \leq 4.5$ ) and a water content of 30%, aluminum is soluble, with expected concentrations of approximately 3.3 mM for an addition of 1  $\mu\text{mol}$  of Al gfw of soil<sup>-1</sup>. Since acidic soils often contain millimolar levels of dissolved aluminum (42), the amounts of aluminum added during this study and the responses to them are ecologically significant.

Although exogenous aluminum inhibits atmospheric methane consumption, the specific causes of inhibition are complex and include several factors. For example, exogenous aluminum can decrease methane uptake by decreasing the soil pH (Fig. 1A and B 5). However, since sulfuric acid inhibits activity less than equivalent amounts of aluminum sulfate inhibit activity and since aluminum is notably more inhibitory than sulfuric acid at a given soil pH (Fig. 1C), pH changes only partially account for inhibition by exogenous aluminum.

Based on the responses of several microbial taxa, including methanotrophs (P. Milligan and G. M. King, unpublished data), aluminum toxicity for methanotrophs in soils likely involves a variety of direct effects. These may include changes in membranes, disruption of enzyme activities, and decreased ATP synthesis (42). In addition, Gullege and Schimel (21) have suggested that certain cations, e.g.,  $\text{K}^+$ , may decrease methanotrophic activity in soils by some general, but unspecified, mechanism that may apply to aluminum. However, the absence of significant  $\text{Na}^+$  or  $\text{K}^+$  inhibition in cultures, in contrast to distinct inhibition by aluminum and ammonium (32; Milligan and King, unpublished data), suggests that aluminum inhibition in soils arises from element-specific phenomena that cannot be readily controlled for or estimated by comparisons with other cations.

The effects of exogenous aluminum may depend in part on the form of aluminum added and on the anionic regime in a given soil solution since aluminum chloride appears to be more inhibitory than nitrate or sulfate salts (Fig. 2). Similar differences have been reported for chloride, nitrate, and sulfate salts of ammonium and other cations (21, 32). Gullege and Schimel (21) have argued that differential anion effects for  $\text{K}^+$  and ammonium reflect an unspecified toxicity of chloride per se. However, since ammonium chloride salts are no more inhibitory than sulfate salts in pure cultures (32), differential sensitivity of soil methanotrophy to various anions may result from interactions between anions and cations that are expressed in soils but not in cultures. Such interactions include but are not limited to the effects of ion pairing on ion sorption and desorption and cell uptake (32). Regardless, aluminum chloride salts should not be avoided in future studies unless chloride is not an important component of the soil solution in the system being examined. For terrestrial systems affected by acid precipitation, nitrate and sulfate salts may be most appropriate; for systems with a maritime influence, a mixture of chloride and other salts may be required.

Addition of chelated aluminum rather than aluminum salts appears to have little impact on toxicity. This may indicate that soil methanotrophs are equally sensitive to dissolved inorganic aluminum species and low-molecular-weight complexed species. Alternatively, aluminum speciation and toxicity in Maine forest soils may be dominated by naturally occurring ligands in fulvic and humic acid fractions of the soil organic matter, the significance of which is not affected by exogenous citrate. Since the total soil organic matter content (about 5% or 2 mmol of C gfw of soil<sup>-1</sup> [1]) vastly exceeds the amount of citrate added

in this study ( $\leq 1 \mu\text{mol gfw of soil}^{-1}$ ), some redistribution of exogenous aluminum among humic and fulvic acids is expected. The extent to which this occurs and controls aluminum toxicity merits further attention.

Although citrate additions do not affect aluminum toxicity significantly in Maine forest soils, the extent of aluminum toxicity in general depends on the methane concentrations to which soils are exposed (Fig. 3 and 4). The nature of this dependency (that is, increasing inhibition with increasing methane concentrations followed by a decrease in toxicity at elevated levels [ $>1,000 \text{ ppm}$ ]) is similar to patterns observed for ammonium toxicity in soils and methanotroph cultures (30, 31, 45). However, the relationship between methane concentration and ammonium inhibition can be explained by competitive effects of ammonium at the level of methane monooxygenase and noncompetitive effects resulting from intracellular hydroxylamine and nitrite production (30, 31). No such explanation is obvious in the case of aluminum. Similarly, increasing inhibition with increasing methane concentrations has been reported for potassium salts in forest soils (21), but since no such trend occurs in methanotroph cultures (32), a physiological explanation for the soil results is uncertain.

For Maine forest soils, increased aluminum (and perhaps potassium) inhibition at elevated methane concentrations may be attributed in part to desorbed ammonium (Fig. 7), which elicits responses comparable to those observed for cultures (31). The extents of desorption observed in this study and previously indicate that cation additions can increase soil water ammonium concentrations by 0.5 to 1 mM, levels that clearly cause inhibition (1, 32, 45). Nonetheless, the mechanisms of increased ammonium and nonammonium salt toxicity warrant further study with soil and culture models since decreases in atmospheric methane consumption capacity represent positive feedback on methane accumulation and greenhouse warming (30).

Results of kinetic analyses indicate that soil methane consumption is especially sensitive to exogenous aluminum but that the inhibition patterns are complex. Decreases in  $V_{\text{max}}$  (Fig. 5) presumably arise from direct effects of aluminum added at relatively low concentrations (0.01 to 0.4  $\mu\text{mol gfw of soil}^{-1}$ ), since pH and the concentrations of ammonium and other cations change to only minor extents at these levels. The apparent  $K_m$  also decreases with low levels of added aluminum, which contrasts with the increase in apparent  $K_m$  (Fig. 6) observed for ammonium added at 0.3  $\mu\text{mol gfw of soil}^{-1}$ . However, at higher aluminum and ammonium concentrations (e.g.,  $>0.4$  and 1  $\mu\text{mol gfw of soil}^{-1}$ , respectively) changes in  $V_{\text{max}}$  and apparent  $K_m$  likely involve multiple phenomena, including processes previously documented in cultures (31) as well as interactions among various ionic species.

Due to the solubility of aluminum at pH values of  $<4.8$  and the widespread acidification of soils resulting from anthropogenic disturbances, aluminum mobilization may play a significant and increasingly important role in the dynamics of soil-atmosphere methane exchanges. In particular, aluminum mobilization may further exacerbate inhibition of atmospheric methane consumption caused by changes in other parameters (e.g., ammonium, pH) and contribute to a greater global warming potential for methane.

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