

# METHANE AND METHANOTROPHY IN TEXAS AQUIFERS

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## INTRODUCTION

Naturally-occurring methane-oxidizing bacteria (methanotrophs) have been shown to degrade the halogenated hydrocarbons that contaminate many groundwaters (Wilson and Wilson, 1985; Fliermans et al., 1988; Little et al., 1988). Despite their importance, there are few studies of methanotroph distribution and abundance in the terrestrial subsurface, especially for deep pristine aquifers.

Many Texas groundwaters contain significant to abundant methane that can serve as a substrate for methanotrophs. Previous study of methane-rich groundwaters from the Claiborne group aquifers in east-central Texas has shown that methane is derived from bacterial degradation of sedimentary organic matter within the aquifer (Grossman et al., 1989). To evaluate whether this methane supports the activity of methanotrophs, we have performed geochemical, isotopic, and microbiological analyses of groundwaters from aquifers in central and east-central Texas.

## MATERIALS AND METHODS

Water was collected from wells in the Eocene sands and shales of the Yegua, Sparta, Queen City, and Wilcox-Carrizo aquifers in east-central Texas, and in the Cretaceous carbonates and sands of the Edwards and Trinity aquifers in central Texas (Figure 1). The wells sampled were municipal wells or monitoring wells of the U. S. Geological Survey and ranged in depth from 54 to 1032 m. Sampling and analytical methods are discussed in detail in Zhang (1994). Briefly, wells were pumped for at least one hour before field measurements and sampling. Temperature, pH, dissolved oxygen, H<sub>2</sub>S, and total dissolved iron were measured at the well site. Nitrate was measured in the laboratory by photometry. Dissolved methane concentrations and <sup>13</sup>C/<sup>12</sup>C ratios were measured using the methods discussed in Grossman et al. (1989). <sup>13</sup>C/<sup>12</sup>C ratios are reported as per mil (‰) deviation from the PDB standard.

For enumeration of methanotrophs, a three-tube most probable number (MPN) method was employed using the American Type Culture Collection #1306 medium (Gherna, et al., 1989). One ml of groundwater (in dilutions of 0, 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>) was injected aseptically into each of four 15 ml Hungate tubes containing 5 ml media. Three tubes were used for cell enumeration, and the fourth was autoclaved and served as a killed control. Culture tubes were evacuated and gassed with pure N<sub>2</sub> at least twice after inoculation to purge air and dissolved gases from the groundwater sample. Culture tubes were then gassed with 20% O<sub>2</sub>/N<sub>2</sub>, and 5 ml of 99% methane was added as the sole carbon source. Control tubes were processed the same way as culture tubes. Tubes were incubated at constant temperatures from two to 16 weeks. Samples with ambient temperatures below 30°C were incubated at 27°C, and those with ambient temperatures above 30°C were incubated at 37°C. Methanotrophic activity was determined by both visual evidence and CO<sub>2</sub> production detected on a gas chromatograph with a thermoconductivity detector (Zhang, 1994).

## RESULTS AND DISCUSSION

Texas deep groundwaters are characterized by low concentrations of oxygen and nitrate and variable contents of dissolved methane (Figure 2). Dissolved oxygen is below 25 μM and nitrate is below the detection limit (1.6 μM) for most water samples analyzed (Zhang, 1994). Dissolved methane concentrations range from below the detection limit (0.01 μM) to 1630 μM. High methane contents are restricted to aquifers in the Eocene sediments of east-central Texas (Figure 2).

Where methane is abundant, its  $\delta^{13}\text{C}$  is low ( $< -50\text{‰}$ ). Conversely, high  $\delta^{13}\text{C}$  values ( $> -35\text{‰}$ ) for methane are only associated with low methane concentrations ( $< 5 \mu\text{M}$ ).

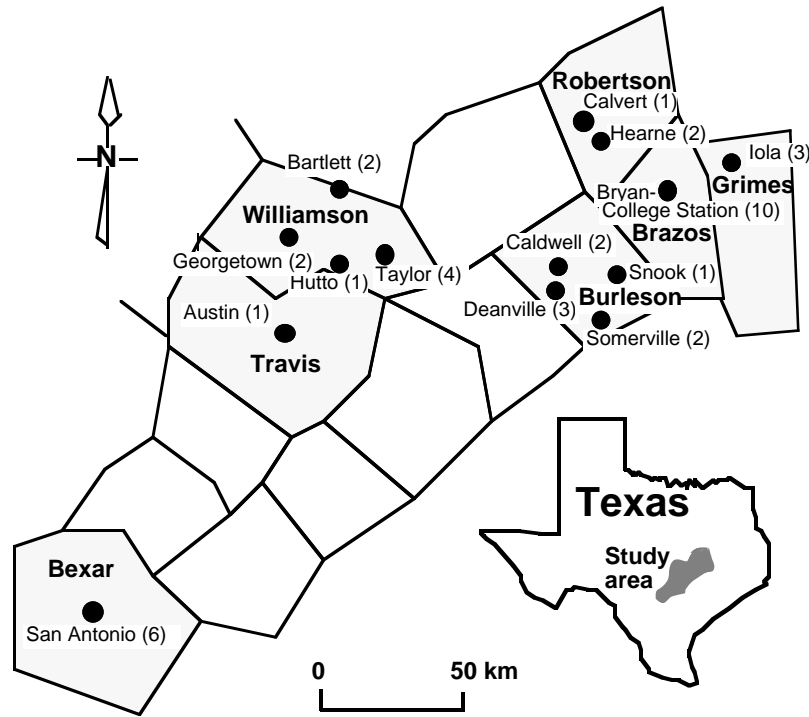


Fig. 1. Location of wells sampled. Number of different wells sampled shown in parentheses.

The MPN values of methanotrophs range from 0 to 460 cells/ml (Figure 3). Samples with abundant methanotrophs ( $> 20$  cells/ml) were common for east-central Texas groundwaters, but not for central Texas groundwaters.

*Origin and Oxidation of Methane.* The  $\delta^{13}\text{C}$  value of methane depends on its origin, the  $\delta^{13}\text{C}$  of the original carbon source, and whether the methane has been modified by oxidation. Thermogenic methane generally has  $\delta^{13}\text{C}$  values between  $-50\text{‰}$  and  $-25\text{‰}$ , whereas microbial methane has values less than about  $-50\text{‰}$  (Figure 2; Schoell, 1980). Methanotrophs preferentially oxidize  $^{12}\text{CH}_4$ , leaving the residual methane enriched in  $^{13}\text{C}$  (Barker and Fritz, 1981). High methane contents are restricted to the aquifers of east-central Texas probably because of the higher organic carbon content of the sediments. The fact that abundant methane is associated with low  $\delta^{13}\text{C}$  values supports a microbial origin for the gas. There is no evidence that thermogenic methane intrudes into these aquifers. Methane that occurs in low concentration has  $\delta^{13}\text{C}$  values ranging from  $-60$  to  $-9\text{‰}$ . Methane with  $\delta^{13}\text{C}$  values less than  $-50\text{‰}$  is likely unmodified and reflects low production rates or mixing. Methane with  $\delta^{13}\text{C}$  values greater than  $-50\text{‰}$  is likely the residual after methane oxidation.

*Factors controlling the distribution and abundance of methanotrophs in Texas groundwaters.* There is a significant correlation between methanotroph abundance and methane concentration (Figure 3). High numbers of methanotrophs are largely found in the sand-shale aquifer system in east-central Texas because that is where methane-rich waters are found. Studies of methanotrophs in marine and lacustrine habitats also show that methanotroph abundance and distribution are related to methane concentrations (Hanson and Wattenberg, 1991).

Waters with  $^{13}\text{C}$ -enriched methane tend to have low methanotroph abundances. The  $^{13}\text{C}$ -enriched methane is likely the product of past oxidation. On the other hand, waters with abundant methanotrophs (and presumably the most methanotrophic activity) contain  $^{13}\text{C}$ -depleted methane,

suggesting that the proportion of methane that has been consumed is small and that oxidation rates are slow relative to the size of the methane reservoir.

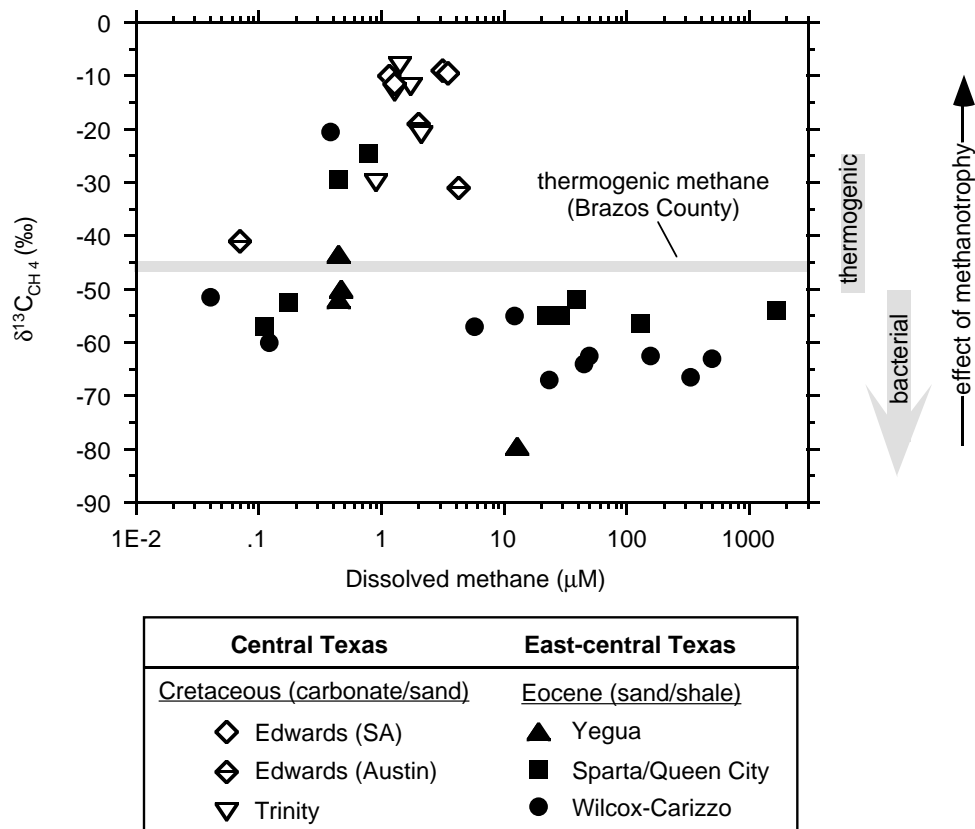


Fig. 2. Carbon isotopic composition versus dissolved methane concentration for central and east-central Texas groundwaters.

It is unclear whether methanotroph abundances reflect aquifer populations or populations at the well. Bacteria tend to live attached to sediment. Cells in groundwater are either planktonic or have detached with pumping (Kölbl-Boelke et al., 1988; Hirsch and Rades-Rohkohl, 1988). Zhang (1994) found that in waters with high methanotroph counts, counts decrease with pumping over a period of 20 or more hours. Turbidity also decreases with pumping, and it is possible that a decrease in counts with time reflects a decrease in suspended sediment with attached cells. On the other hand, culture experiments demonstrate that methanotrophs can grow under the low oxygen conditions of the aquifers (Zhang, 1994). Future studies will examine methanotroph abundances in aquifer sediments.

This study shows that methanotrophs are common in the deep subsurface and their distribution is related to groundwater methane concentration. Because many microaerophiles including methanotrophs are capable of fixing  $N_2$  and utilizing a scarce supply of oxygen (Rudd and Taylor, 1980; Benoit and Phelps, 1990), they may have an advantage competing with other heterotrophic bacteria in the oligotrophic and oxygen depleted environments of the deep subsurface.

### ACKNOWLEDGMENTS

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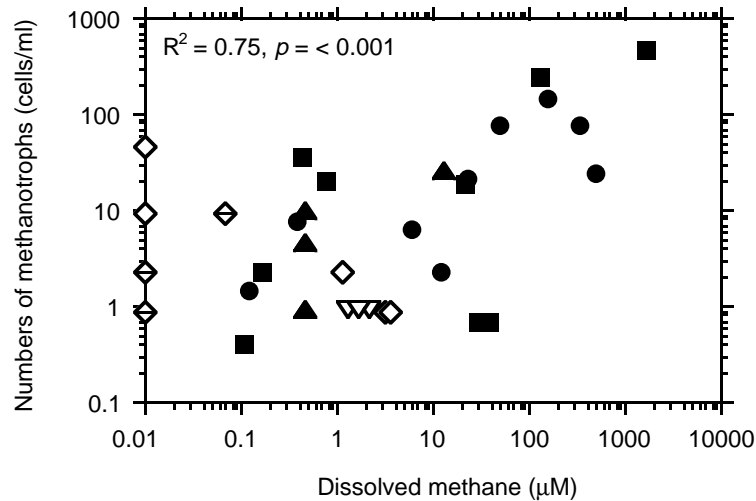


Fig. 3. Methanotroph abundance as a function of methane concentration of groundwaters from Texas aquifers. Samples with methane concentrations below the detection limit (0.01 µM) are treated as having 0.01 µM for statistical and graphing purposes. Symbols same as in Figure 2.

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