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Final Report

Title: **Root-associated methane oxidation and methanogenesis: key determinants of wetland methane emissions**

Report type: **Final**

Principal investigator: **G.M. King**

Project period: **August, 1993-August, 1997** (includes 1 yr no-cost extension)

Grantee institution: **University of Maine, Orono, ME 04469**

Grant number: **NAGW-3746**

Summary or research accomplishments

During the award period, we have assessed the extent and controls of methane oxidation in north temperate wetlands. It is evident that wetlands have been a major global source of atmospheric methane in the past, and are so at present. It is also evident that microbial methane oxidation consumes a variable fraction of total wetland methane production, perhaps 10%-90%. Methane oxidation is thus a potentially important control of wetland methane emission. Our efforts have been designed to determine the extent of the process, its controls, and possible relationships to changes that might be expected in wetlands as a consequence of anthropogenic or climate-related disturbances.

Methane oxidation in wetlands occurs at two primary loci: the sediment-water (or air) interface and in the rhizosphere or rhizoplane of rooted vegetation. In earlier work, we have addressed the rates and controls of methane oxidation at the sediment-water interface. Methane oxidation at this locus appears controlled primarily by the availability of molecular oxygen, although in certain systems methane supply might also become a limiting factor. Oxygen availability is limited in turn by the supply of organic matter (controlling respiratory demand), benthic photosynthesis (controlling both organic supply and oxygen) and water table dynamics (controlling oxygen transport) among other parameters. Short-term as well as long-term shifts in these parameters can alter oxygen availability and the extent of methane oxidation. For example, a number of studies have shown that sustained decreases in the water table that result in desaturation of wetland peats promote increased oxidation. In contrast, short-term water table fluctuations on a scale of mm-cm and days can have the opposite effect in saturated peats.

Current work, has emphasized controls of methane oxidation associated with rooted aquatic plants. As for the sediment-water interface, we have observed that oxygen availability is a primary limiting factor. Our conclusion is based on several different lines of evidence obtained from in vitro and in situ analyses.

First, we have measured the kinetics of methane oxidation by intact plant roots harboring methane-oxidizing bacteria, as well as the kinetics of the methanotrophs themselves. Values for the half-saturation constant (apparent K_m) are approximately 5 μM. These values are roughly equivalent to, or much less than porewater methane concentrations, indicating that uptake is likely saturated with respect to methane, and that some other parameter must limit activity. Methane concentrations in the lacunar spaces at the base of plant stems are also comparable to the half-saturation constants (when expressed as equivalent dissolved concentrations), providing further support for limitation of uptake by parameters other than methane.

In addition, we have observed substantial variations among plant taxa in maximum potential methane oxidation by root-associated methanotrophs. In many cases, multiple taxa have been obtained from sites where they grow contiguously, and were exposed to similar regimes of porewater methane. Substantial differences have also been noted among root types (e.g., fine, feeder roots versus tap or anchor roots), even though exposures to dissolved methane have been similar. Some of the differences among roots and among plant taxa have been accounted for by differences in root surface area. However, it remains evident that the large unexplained differences must arise from parameters other than methane availability.

Second, data from an in situ analysis shows no correlation between rates of methane oxidation and total potential methane emission, which is a measure of methane availability. The absence of a correlation indicates that the supply of methane per se controls neither the extent of oxidation (percent of total potential emission), nor the absolute rate of activity. Interestingly, the extent of oxidation generally appears higher at the peat surface (average values > 50%) than in the sub-surface rhizosphere/rhizoplane (average values about 25%). These results also support a limited role for methane as a control of oxidation, since surface methane oxidation is confined to the upper 5-10 mm (based on in situ assays of oxygen penetration) where methane concentrations are least.

Third, we have conducted *in vitro* assays using intact plants in split chambers that allowed manipulation of the medium in which the roots were immersed. Manipulations included incubations with saturating levels of oxygen (> 200 µM), sub-oxic levels (<20 µM), and anoxic conditions (created and sustained with a highly efficient microbial oxygen sink). Data from these assays clearly indicated that the supply of oxygen was an important control of methanotrophic activity, and that oxygen is perhaps the overriding control in most cases.

In addition, our *in vitro* chamber analyses have also revealed substantial differences in the ability of different plant species to oxygenate their rhizospheres and to support methanotrophic activity. We have compared rates of methane oxidation and patterns of rhizosphere oxygenation by two common wetland macrophytes, the bur-weed, *Sparganium eurycarpum*, and the pickerelweed, *Pontederia cordata*. Though the roots and rhizomes of these plants appear similar morphologically, oxygen loss from *S. eurycarpum* is much more limited than from *P. cordata*. This likely explains the lower rates of in vitro methane oxidation associated with *S. eurycarpum.* We have confirmed the chamber observations with a field analysis that showed a greater percentage of methane oxidation for *P. cordata* than for *S. eurycarpum*.

However, the observed patterns of rhizosphere oxygenation were enigmatic in an important respect. Methanotrophic activity (an obligately aerobic process) for both taxa was significantly greater on fine (diameters << 1 mm) than smooth (diameters ≥ 1 mm) roots, but virtually all oxygen loss occurred through the latter. One hypothesis for this pattern is that oxygen loss occurs in older roots (e.g., smooth roots) due to changes in root wall porosity, with a portion of the loss supporting rhizoplane activity; in contrast, little (if any) oxygen is lost from the younger (fine) roots, but these roots harbor populations of methanotrophs that are more active within than outside of roots. Assays of root methane oxidation before and after rinses with a solution of sodium dodecyl sulfate (which removes bacteria from the root surface) indicate that populations of methanotrophs exist inside of roots; however this hypothesis remains to be fully tested.

In general, these results have a number of implications beyond providing an estimate of the extent of root methanotrophy and its proximate controls. Of particular significance is the fact that wetland macrophytes differ markedly in their capacity to support methane oxidation. Since increasing pCO_2 and regional climate change can be expected to promote unpredictable successional changes in species composition, the extent of methane oxidation, and therefore limits on methane emission, cannot be reliably predicted. The major role of oxygen availability also increases the unpredictability of future rates of methane consumption. This is because temperature and plant growth status can affect oxygen transport and loss from roots. Finally, the distribution of root types, e.g., smooth versus fine, is sensitive to a variety of edaphic factors. Nutrient limitation and other stresses stimulate production of fine roots, while nutrient enrichment has the opposite effect. Eutrophication of wetlands, which is occurring globally could therefore decrease fine roots and associated methanotrophy.

During the course of our research, we also addressed a number of other issues, including:

a) the capacity of methanotrophs to survive extended periods of anoxia; we assessed the physiological mechanisms which allow methanotrophs to persist in the absence of oxygen, a condition common in wetlands;

b) the attributes of root-associated methanotrophs; we determined the nature of methanotroph diversity and the specificity of associations with roots;

c) controls of atmospheric methane consumption by forest soils, including the response of soils to nitrogen fertilization.

Our work has been presented at a number of national/international meetings and published in major journals as summarized below.

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