Short communication

Methane oxidation and phospholipid fatty acid composition in a podzolic soil profile

Ingvar Sundh\textsuperscript{a,*}, Gunnar Börjesson\textsuperscript{b}, Anders Tunlid\textsuperscript{c}

\textsuperscript{a}Department of Microbiology, Swedish University of Agricultural Sciences, PO Box 7025, SE-750 07 Uppsala, Sweden
\textsuperscript{b}Department of Water and Environmental Studies, Linköping University, SE-581 83 Linköping, Sweden
\textsuperscript{c}Department of Microbial Ecology, Lund University, SE-223 62 Lund, Sweden

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Abstract

We compared methane oxidation activity in laboratory incubations of samples from a podzolic soil profile to the microbial community structure of the soil, determined as the content and composition of phospholipid fatty acids (PLFAs). The abundances of two fatty acids considered unique for methanotrophs (and the abundances of all other quantified PLFAs) were very weakly related to methane oxidation. This is in contrast to the situation in environments with much higher methane supply, indicating that these fatty acids should not be used as biomarkers for methanotrophs in upland forest soils.

Keywords: Methane oxidation; PFLA composition; Podzols; Microbial community structure

1. Introduction

Microbially-mediated oxidation makes most aerated mineral soils act as sinks for atmospheric methane, a gas making a substantial contribution to the anthropogenic greenhouse effect (Rodhe, 1990). However, it has not been established which organisms are responsible for the oxidation of methane in mineral soils. Notably, known isolates of methane-oxidizing bacteria are not able to grow on atmospheric methane concentrations (currently ca. 1.8 \textmu mol l\textsuperscript{-1}) (Benstead et al., 1998; Conrad, 1984). Ammonium-oxidizing bacteria (Bedard and Knowles, 1989), methanotrophs with high affinities for methane (Bender and Conrad, 1992; Dunfield et al., 1999) and mixotrophic methanotrophs (Benstead et al., 1998; Jensen et al., 1998) have been proposed as possible candidate organisms.

Many methane-oxidizing bacteria contain unusual phospholipid fatty acids (PLFAs) of 16 (Type I methanotrophs) or 18 (Type II) carbons in length (Bowman et al., 1993). The abundances of these unusual PLFAs have been used to estimate the biomass distribution of Type I and II methanotrophic bacteria in environments well supplied with methane (Börjesson et al., 1998; Nichols et al., 1987; Sundh et al., 1995).

In the present study, we looked for correlations between methane oxidation activity and the microbial community structure (determined from analysis of PLFAs) in samples from a typical podzolic forest soil profile.

2. Materials and methods

Soil was collected in summer in a fairly dense spruce stand (\textit{Picea abies}) on a forested moraine ridge, close to the Swedish University of Agricultural Sciences in Uppsala in the temperate zone of Sweden (59°49’N, 17°39’E). The soil is a Dystrochrept (according to the Soil Taxonomy system), i.e. it is an acidic but not fully...
developed podzol in a cool climate. Roughly, the O (organic) horizon extends from the surface to 5 cm depth, the A (eluviation) horizon from 5 to 20 cm, and the B (illuviation) horizon from 20 to 40 cm. Some basic characteristics of the soil are given in Table 1. Four cores (diameter 33 mm) down to 20 cm were taken within an area of approx. 0.3 m². The cores were cut into 2-cm sections and the four replicates from each depth pooled and taken to the laboratory.

Soil used for measurements of the methane oxidation potential (the oxidation rate at saturating methane and oxygen concentrations, \( V_{\text{max}} \)) was collected at a later date. This time, bulk soil from the 0–5, 5–10, and 10–20 cm intervals was collected.

For measurements of methane uptake at atmospheric concentration, duplicate 5-g (O horizon) or 10-g portions were transferred to serum bottles (118 ml), which were sealed and stored at 15°C overnight. The bottles were then equilibrated with air and incubated at 20°C. Samples for methane analysis were taken immediately and at intervals during ca. 7 h. The methane concentration in the bottles was analyzed by gas chromatography, principally according to Örlygsson et al. (1993). The consumption of methane followed first-order kinetics and oxidation rates at the initial atmospheric concentration were calculated from exponential equations.

The measurements of \( V_{\text{max}} \) were similar, but methane was added to ca. 10, 100, and 1000 \( \mu l \) \( l^{-1} \) to parallel bottles, so that zero-order kinetics (linear concentration decrease of methane) was obtained. In a separate series, de-ionized water was added, and the bottles were subsequently shaken (200 rev min\(^{-1}\)) during incubation. The incubation time was 22 h. Rates were slightly higher with water addition, and \( V_{\text{max}} \) was calculated by linear regression for methane consumption in water-amended bottles with 1000 \( \mu l \) CH\(_4\) \( l^{-1} \).

The methods for PLFA analysis were described by Börjesson et al. (1998) and references therein. Forty different PLFAs were identified and quantified.

### 3. Results and discussion

The oxidation rate at atmospheric concentration was zero at the surface but increased to a subsurface maximum around 15 cm, indicating highest biomass of methanotrophs at this depth (Fig. 1). A subsurface maximum is very typical for temporal and boreal forest soils (e.g. Roslev et al., 1997; Whalen et al., 1992).

In the \( V_{\text{max}} \) estimates, there was no detectable oxidation down to 10 cm, regardless of methane concentration or water addition and agitation. However, at 10–20 cm oxidation was substantial (Table 1), and of the same order of magnitude as in other reports (Bender and Conrad, 1992; Benstead and King, 1997; Roslev et al., 1997; Whalen et al., 1992).

None of the PLFAs quantified in this soil was well correlated with oxidation activity. Interestingly, the abundance of the two \( \omega8 \) fatty acids that worked so well as biomarkers for methanotrophs in peatlands and landfills (Börjesson et al., 1998; Sundh et al., 1995), was totally unrelated to methane oxidation activity (Fig. 1). This indicates that methanotrophs are not a likely major source of these fatty acids.

Based on the calculations below, however, we conclude that the dissimilarity between the distributions of oxidation and the \( \omega8 \) PLFAs in this soil profile is not absolute proof that “classical”, low affinity methanotrophs similar to known isolates cannot be responsible for the oxidation. Based on \( V_{\text{max}} \) (46 pmol g\(^{-1}\) d.w. soil min\(^{-1}\)) we calculated the corresponding, expected, amounts of the \( \omega8 \) PLFAs if “classical” methanotrophs were responsible for the oxidation. The following assumptions were made: (1) The \( V_{\text{max}} \) of methanotrophic bacteria is 500 nmol mg\(^{-1}\) d.w. cells min\(^{-1}\) (Carlson et al., 1991; Sheehan and Johnson, 1992).

### Table 1

<table>
<thead>
<tr>
<th>Horizon (cm)</th>
<th>Moisture (% of f.w.)</th>
<th>Organic matter (% of d.w.)</th>
<th>pH(^a,b)</th>
<th>( V_{\text{max}}) ( a ) (pmol CH(_4) g(^{-1}) d.w. min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>62</td>
<td>87</td>
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<tr>
<td>2–4</td>
<td>43</td>
<td>65</td>
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<td>4–6</td>
<td>21</td>
<td>39</td>
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<td>6–8</td>
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<td>8–10</td>
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<td>16–18</td>
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<td>18–20</td>
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</table>

\( a \) pH and \( V_{\text{max}} \) were measured on bulk soil samples from 0–5, 5–10, and 10–20 cm depth.

\( b \) pH was measured in soil suspensions in de-ionized water.
1971). (2) Methanotrophs contain 100 nmol PLFA mg\(^{-1}\) d.w. cells (Nichols et al., 1985; White et al., 1979a). (3) Half of the PLFAs in methanotrophs are \(\omega 8\) (Bowman et al., 1993). The calculations revealed that we could expect ca. 5 pmol \(\omega 8\) PLFA g\(^{-1}\) d.w. soil, which is less than 10% of the actual concentrations in the mineral horizon (Fig. 1). Obviously, these amounts of \(\omega 8\) PLFAs in methanotrophs may be impossible to distinguish, if there are additional sources of these PLFAs.

Possible alternative sources of the PLFAs are dead cell material in organic detritus, or other living microorganisms. Regarding phospholipids in dead cell material, they are degraded relatively rapidly in sediments (White et al., 1979b), but perhaps more slowly in soils (e.g. Frostegård et al., 1996). With the exception of some Bacillus species (Fulco, 1969), we know of no examples of the presence of 16:1\(\omega 8\) or 18:1\(\omega 8\) in organisms other than methanotrophic bacteria. In methanotrophs, however, the \(\omega 8\) unsaturation always occurs together with unsaturation in other positions (particularly \(\omega 7\) and \(\omega 9\)), very common in many other eubacteria. In this soil profile, the \(\omega 8\) isomers were typically less than 1% of the total amounts of 16 and 18 carbon monounsaturated PLFAs. Additionally, their concentrations were positively correlated with those of several of the other isomers. Thus, maybe the main source of the \(\omega 8\) isomers was trace amounts in non-methanotrophic organisms containing monounsaturated 16- and 18-carbon PLFAs. The reason that they have not been found in other organisms may be that no-one has been actively looking for them.

From our results, we conclude that the major part of the \(\omega 8\) PLFAs in this podzolic forest soil does not originate in methanotrophic bacteria. Therefore, these PLFAs should not be used as biomass indicators of methanotrophs in these types of soil, and maybe other upland forest soils.

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