The active methanotrophic community in a wetland from the High Arctic

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Summary

The dominant terminal process of carbon mineralization in most freshwater wetlands is methanogenesis. With methane being an important greenhouse gas, the predicted warming of the Arctic may provide a positive feedback. However, the amount of methane released to the atmosphere may be controlled by the activity of methane-oxidizing bacteria (methanotrophs) living in the oxic surface layer of wetlands. Previously, methanotrophs have been isolated and identified by genetic profiling in High Arctic wetlands showing the presence of only a few genotypes. Two isolates from Solvatnet (Ny-Ålesund, Spitsbergen; 79°N) are available: Methylobacter tundripaludum (type I) and Methylocystis rosea (type II), raising the question whether the low diversity is a cultivation effect. We have revisited Solvatnet applying stable isotope probing (SIP) with 13C-labelled methane. 16S rRNA profiling revealed active type I methanotrophs including M. tundripaludum, while no active type II methanotrophs were identified. These results indicate that the extant M. tundripaludum is an active methane oxidizer at its locus typicus; furthermore, Methylobacter seems to be the dominant active genus. Diversity of methanotrophs was low as compared, e.g. to wetland rice fields in the Mediterranean. This low diversity suggests a high vulnerability of Arctic methanotroph communities, which deserves more attention.

Introduction

Methane is an effective greenhouse gas with the second largest radiative forcing after carbon dioxide (Intergovernmental Panel on Climate Change, 2007). Climate change is expected to strongly influence the Arctic regions by an over-proportional warming (Arctic Climate Impact Assessment, 2004). This effect could not only transform the Arctic tundra from a carbon sink to a carbon source (Schuur et al., 2008), but also increase the source strength of methane (Friborg et al., 2003). In the active layer above permafrost, methanogenesis is the dominating terminal process of anaerobic decomposition of organic matter (Wagner et al., 2005). However, only part of the methane produced is emitted: methanotrophs serve as a biological filter significantly reducing methane emissions from wetlands (Reeburgh et al., 1993; Conrad, 2009). Up to 95% of the methane produced in anoxic soils may be oxidized before reaching the atmosphere (Frenzel et al., 1990). Hence, even small changes in oxidation capacity may be of global importance, if key areas like the Arctic are concerned.

Based on physiology, biochemistry and ultrastructure, aerobic proteobacterial methanotrophs have been divided into two subgroups, type I and type II (Trotsenko and Murrell, 2008). This traditional classification corresponds quite well to the phylogenies of the 16S rRNA – and pmoA genes. The latter encodes a subunit of the particulate methane monoxygenase, the key enzyme catalysing the first step in methane oxidation. More recently, extremophilic methanotrophic Verrucomicrobia have been discovered representing a distinct lineage apart from the canonical methanotrophs (Dunfield et al., 2007; Op den Camp et al., 2009). Most methanotrophs use methane as their sole carbon and energy source (Trotsenko and Murrell, 2008), but a few strains have been found capable growing on C2 compounds (Dedysh et al., 2005; Belova et al., 2010; Dunfield et al., 2010).

In general, type I methanotrophs dominate in the Arctic (Wartlainer et al., 2003; Liebner and Wagner, 2007). From cold habitats, various methanotrophs have been described: Methylobacter psychrophilus is a psychrophilic isolate from the polar Ural (Omelnchenko et al., 1996; Tourova et al., 1999); unfortunately, this culture has been lost (P. Kaempfer, pers. comm.). Methylomonas scandinavica is a psychrotolerant from deep igneous rock with an optimum growth at 15°C (Kalyuzhnaya et al., 1999). Two type II methanotrophs with growth optima at low temperatures are known, the psychrophilic Methylosphaera hansoni from Antarctica and the psychrotolerant Methylocella tundrae from Siberian peatlands (Bowman et al., 1997;
Dedysh et al., 2004). From Svalbard two psychrotolerant methanotrophs were isolated, Methylobacter tundripaludum (type I) and Methylocystis rosea (type II) with optimal growth temperatures of 23°C and 27°C, respectively (Wartiainen et al., 2006a,b).

In the period between 2001 and 2006, M. tundripaludum was repeatedly re-isolated in our laboratory from its locus typicus, Solvatnet, Svalbard (data not shown). However, cultivation may select for particular ecotypes that are not necessarily active in situ. Stable isotope probing (SIP) allows studying active organisms in complex environmental samples (Murrell and Radajewski, 2000; Radajewski et al., 2000; McDonald et al., 2005).

Exploiting the ability of methanotrophs to use methane both for catabolism and anabolism, 13CH4 (‘heavy methane’) has been used repeatedly to study active methanotrophic communities. RNA-SIP (Manefield et al., 2002) targeting a high copy biomarker is the method of choice when growth is slow, e.g. at low temperatures. We focused on in situ summer temperatures of the upper soil layer (8–16°C) that are nevertheless quite moderate in the Arctic, and studied the metabolically active methane oxidizers in peat from Solvatnet by RNA-SIP. This experiment provides essential baseline information on the diversity of an active population, and is crucial for further studies on resistance and resilience of Arctic methanotrophic communities.

Results and discussion

The sampling site (78°55′ N, 11°56′ E) is a wetland surrounding Solvatnet, a pond on the Brøgger peninsula near Ny-Ålesund. It is situated on Spitsbergen, the biggest island of the archipelago Svalbard. Vegetation is composed primarily of mosses and sedges, heavily grazed by barnacle geese (Branta leucopsis) and Svalbard reindeer (Rangifer tarandus platyrhynchus). The active soil layer consists of a peat formed by weakly decomposed mosses. In September 2007, eight cores were taken in lightproof PVC tubes as described previously (Wartiainen et al., 2003). Before the cores were used in the experiment, they were kept at 10°C under permanent illumination mimicking Arctic summer. The top 1.4 cm of the peat wereoxic as revealed by oxygen microprofiles. Thus, three layers were chosen, representing the oxic zone (0–1 cm), the interface (1–2 cm) and the anoxic zone (2–6 cm), further designated layer I, II and III. Replicates were incubated under air, and under air amended with 12CH4 and 13CH4, respectively (Figs 1 and 2).

Interestingly, the peat was capable of oxidizing atmospheric methane. No significant differences between layers II and III were found, with oxidation of 0.43 nmol CH4 (g peat)−1 day−1 and 0.5 nmol CH4 (g peat)−1 day−1, respectively (Fig. 1), while layer I showed the lowest oxidation value with 0.29 nmol CH4 (g peat)−1 day−1. Methane oxidation continued down to at least 0.3 ppmv (0.5 nM). Uptake of atmospheric methane by Arctic soils has been shown repeatedly (Whalen and Reeburgh, 1990; Christensen et al., 1995; Adachi et al., 2006), but the microorganisms responsible for atmospheric methane oxidation in the Arctic are still unknown. While this suggests that Solvatnet might even act as a sink to atmospheric methane, the highest methane oxidation rate is expected at the oxic/anoxic interface contained in layer II. Here, both oxygen...
and methane are available, and methane fluxes from the anoxic peat below should be highest. Methane oxidation at elevated concentrations (1–5% CH₄) started immediately in layer II and III, with an average rate of 7.05 and 3.03 m mol CH₄ (g peat)⁻¹ day⁻¹ respectively (Fig. 2). Rates measured in layer II and III were higher than those reported from the Lena Delta (Siberia; up to 0.17 m mol CH₄ (g soil)⁻¹ day⁻¹ at 1°C, Wagner et al., 2005) reflecting the higher incubation temperature in our experiment. Layer I had a lag phase of approximately 6 days. While oxidation was nearly constant with time in layers II and III, it increased steadily in layer I. Hence, layers II and III were chosen for SIP analysis.

No separation of heavy and light 16S rRNA was achieved after 14 days of incubation with ¹³CH₄. After 28 days, however, T-RFLP patterns changed along the density gradient indicating that label was incorporated into a subpopulation (Fig. 3). Based on these T-RFLP profiles, characteristic light and heavy fractions were chosen to construct clone libraries. A total of 50 16S rRNA gene clones from the light fractions (II: 21 clones; III: 29 clones) were analysed, showing typical soil microbes, belonging mainly to the phyla Firmicutes, Actinobacteria and Acido-
bacteria. No sequences could be affiliated to clades containing methanotrophic bacteria.

In contrast, the clone library from the heavy fractions (II: 26 clones; III: 27 clones) showed a clear predominance of sequences related to sequences of cultivated methanotrophic bacteria; no differences could be observed between layer II and III (Fig. 4). The active community as revealed by ¹³CH₄ RNA-SIP contained four species-equivalent OTUs (operational taxonomic units) clustering within Methylococcaceae (98% similarity; Figs 4 and 5). Most sequences (n = 25) clustered together (SV2) with sequences of Methylobacter sp. T20 (Ren et al., 2000), M. psychrophilus and M. tundripaludum. Hence, the latter is indeed an active player in its locus typicus. The other cluster (SV1, n = 5) had Methylosoma difficile (Rahalkar et al., 2007) as its closest cultivated species. From the few sequences not clustering within clades containing cultivated methanotrophs, other functional relationships became evident: clone sequences from the heavy fraction could be affiliated to sequences of methylotrophic Hyphomicrobiurn sp., suggesting consumption of intermediates leaking out of methanotrophs (Lueders et al., 2006; Osaka et al., 2008). The co-occurrence of labelled

Fig. 3. Characteristic electropherograms of 16S rRNA amplicons representing the light and heavy fractions obtained from the SIP experiment. Top panel: layer II (1–2 cm); bottom panel: layer III (2–6 cm). The exact buoyant densities are given in brackets. Nucleic acids were extracted from 0.3 g of peat ground in liquid nitrogen and processed as described elsewhere (Lueders et al., 2004). Isopycnic centrifugation of RNA was carried out in caesium trifluoroacetate. The gradients were separated into 16 fractions from bottom to top (Noll et al., 2008). From all fractions, the 16S rRNA was amplified by one-step reverse transcription (RT)-PCR (Access QuickTM, Promega) using the primer pair 27f/907r (Lane, 1991). Controls without RT were used to verify RNA purity. For T-RFLP, the forward primer 27f was labelled with FAM (5-carboxyfluorescein) at the 5’ end and the amplicon digested with MspI. T-RFLP analysis was carried out on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) with the GeneMapper software (version 4.0, ABI).
sequences affiliated to sequences of predatory Myxococcales with methanotrophs has been reported previously indicating a common predator-prey relationship in ecosystem as diverse as Arctic wetlands, Mediterranean rice fields, and caves (Hutchens et al., 2004; Murase and Frenzel, 2007).

Although using a cut-off value of 98% similarity for the gene may not exactly reflect the species level, it allows a few comparisons to other environments. In the Lena Delta, a total of five OTUs were found in a clone library of similar size based on DNA and methanotroph-specific assays (Liebner et al., 2009). Using soil samples and enrichments from three different sites on Svalbard, most sequences retrieved from DGGE bands clustered with sequences of *M. tundripaludum* (Wartiainen et al., 2003), but also a few with type II methanotrophs. In contrast, the Mediterranean rice fields in Vercelli (Italy) harbours about 15 genus-equivalent OTUs (Lüke et al., 2010). While the latter estimate is based on *pmoA*, it is a conservative number considering the different substitution rates and distance matrices of 16S rRNA and *pmoA* sequences respectively (Heyer et al., 2002; Degelmann et al., 2010).

Our experiment reflects the conditions during summer when even in the High Arctic temperatures may raise

![Maximum likelihood tree of methanotrophs, based on the 16S rRNA gene.](http://www.arb-silva.de/aligner) Sequences were aligned to their 40 nearest neighbours using SINA (http://www.arb-silva.de/aligner) and imported into ARB (Ludwig et al., 2004), where the alignment was refined manually. Relevant sequences from the SILVA SSURef 100 database (Pruesse et al., 2007) and environmental sequences showing up as close relatives in nucleotide BLAST (GenBank, http://www.ncbi.nlm.nih.gov) were chosen for further analysis (n = 2431). Aligned sequences of more than 1200 bp were used to calculate a ‘backbone’ tree, while shorter sequences were added via quick-add with ARB parsimony using a 50% positional conservation filter. The tree topology was tested with different algorithms: distance matrix (neighbour joining), maximum likelihood (RAxML) and maximum parsimony (PHYLIP) (Felsenstein, 1997; Stamatakis, 2006) and largely consistent. Bootstrap (n = 1000) values ≥ 90% are marked with filled circles. Sequences of active methanotrophs from 13C-RNA fractions were added by quick-add with ARB parsimony. To the right, environmental clusters SV1 and SV2 and OTUs are marked. MOthur (v.1.9.0; Schloss and Handelsman, 2005) was used to group sequences in OTUs (cut-off dissimilarity 2%). Distance bar: 10% sequence similarity; squares: sequences from layer II; diamonds: sequences from layer III. The sequences have been deposited in DDBJ/EMBL/GenBank nucleotide databases under Accession No. HQ534070–HQ534099.
above 10°C at the soil surface. During that period, microbial activities are at maximum, and functioning of methanotrophs will be most important in controlling methane emissions. At lower temperatures, however, other genotypes may be active.

In conclusion, the methanotrophic community in the Arctic, both total and active, is much less diverse than, e.g., in the Mediterranean. Under severely limiting ecological conditions chances are high to cultivate main players, as shown for Solvatnet with *M. tundripaludum* that turned out to be indeed active in its native substrate. Low diversity, however, may have a major drawback: theory predicts that redundancy in diverse communities stabilizes ecosystem functioning. Vice versa, the low diversity of Arctic methanotrophs suggests a certain vulnerability of these communities, and their role in methane cycling deserves increased attention.

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