Molecular biomarkers of methanotrophs in *Sphagnum* peat bogs

J.F. van Winden[1], G-J. Reichart[1], N. Kip[2], H.J.M. Op den Camp[2], J.S. Sinninghe Damsté[1,3]

1. Utrecht University, Department of Earth Sciences, j.vanwinden@geo.uu.nl; P.O. Box 80.021, 3508 TA Utrecht, The Netherlands, ; 2. Radboud University Nijmegen; 3. Royal Netherlands Institute for Sea research (NIOZ)

INTRODUCTION

Fig. 1. *Sphagnum,* peat moss, proliferates in nutrient poor, rain fed, raised bogs. *Sphagnum* exchanges nutrients for protons, acidifying its environment.

Wetlands are the most important natural source for atmospheric methane [1], in spite of the fact that most of the methane produced in a peat bog never reaches the atmosphere. This methane is consumed by methanotrophs, aerobic methane oxidizing bacteria. Molecular probes have shown that such bacteria live as symbionts in *Sphagnum* [2]. The significance of this symbiosis on a global scale is, however, yet unknown. The assessment of this, and of the impact of past and future environmental change on the activity of methanotrophs, is crucial for an accurate understanding of the global carbon cycle.

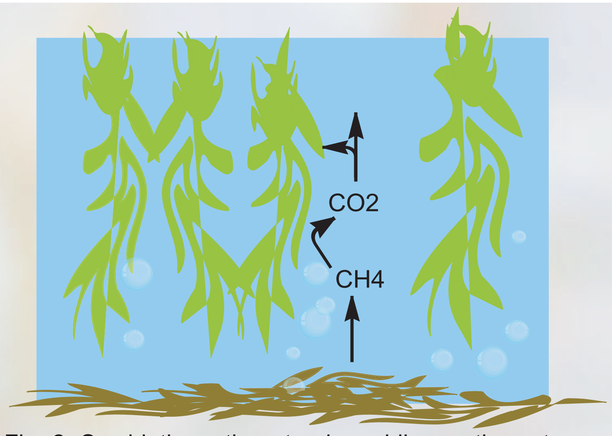

Fig. 2. Symbiotic methanotrophs oxidize methane to carbon dioxide, which is in turn assimilated by *Sphagnum.* This way methane is efficiently recycled.

APPROACH

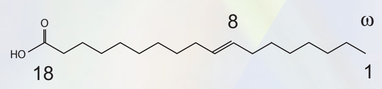

Fig. 1a) C18:1ω8 fatty acid, 18 carbon atoms, 1 double bond at position ω8. Together with C16:1ω8 one of the two fatty acids specific for methanotrophs.

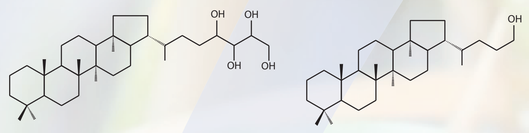

Fig. 2b,c) Bacteriohopanetetrol (2b), common in methanotrophs [5], forms C_{32}-hopanol (2c) after periodic acid treatment, enabling isotope analyses [6].

Sphagnum samples were analysed for molecular biomarkers of methanotrophs. Presence of methanotrophs was confirmed by methane consumption during incubation. Known methanotroph biomarkers include specific mono-unsaturated fatty acids and ^{13}C depleted hopanoids [3], [4].

Methanotrophs can be divided into two genetically distinct groups. Type I methanotrophs use solely methane as their carbon source. Type II methanotrophs use both methane and carbon dioxide as their carbon source, and are dominant in high methane, low nutrient environments [4].

Fig. 3. *Sphagnum* peat bogs have, due to their acidity, a very high carbon accumulation potential. It has been estimated that peatlands store up to one-third of the world's soil carbon [1].

PRELIMINARY RESULTS

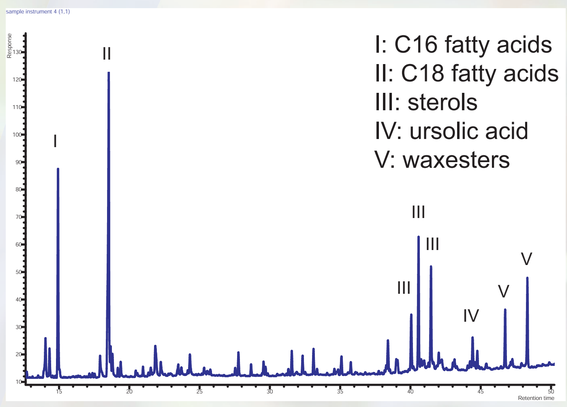

Fig. 4. Total extract lipid chromatogram of *Sphagnum,* methylated and silylated.

C_{32}-hopanol was obtained after treatment with periodic acid, and was found to have a $\delta^{13}C$ value of ~-35‰. The relatively low depletion can be explained by dominance of type II methanotrophs, which use both methane and carbon dioxide.

No diagnostic C18:1ω8 fatty acids were found, but mainly C18:1ω9, C18:1ω7 and C16:1ω7. C18:1 fatty acids were dominant compared to C16:1 fatty acids, indicating prevalence of type II methanotrophs [4]. This is in agreement with cultured acidophilic type II methanotrophs [8] and this is also likely in the acidic, high methane, low nutrient environment.

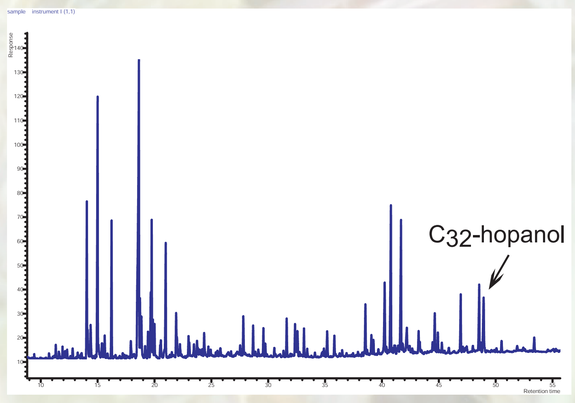

Fig. 5. Polar fraction of extract, treated with periodic acid, methylated and silylated, showing released C_{32}-hopanol.

FURTHER RESEARCH

Fig. 6. The environment of Dewey marsh, Bodmin Moor, UK. All pictures shown on this poster are from this site.

Ongoing research focuses on an inventory of the symbiosis between *Sphagnum* and methanotrophs and on lipid analyses of isolated methanotroph cultures. Incubation of *Sphagnum* with labelled methane is in progress in order to establish the methanotrophic origin of hopanoids and to assess the incorporation of methane-derived carbon dioxide into specific *Sphagnum* lipids. Our ultimate goal is to develop an adequate approach combining biomarkers and stable isotopes, in order to investigate environmental controls of methane cycling in ancient peats.

References: [1] Smith, L.C., MacDonald, G.M., Velichko, A.A., Beilman, O.K., Frey, K.E., Kremenetski, K.V., Sheng, Y., 2004, Science 303, pp. 353-356; [2] Raghoebarsing, A.A., Smolders, A.J.P., Schmid, M.C., Rijpstra,W.I.C., Wolters-Arts M., Derksen, J., Jetten M.S.M., Schouten, S., Jaap S. Sinninghe Damsté, J.S., Lamers, L.P.M., Roelofs, J.G.M., Op den Camp, H.J.M. , Strous, M., 2005, Nature, Vol. 436, no. 25, pp. 1153-1156; [3] Jahnke, L.L., Summons, R.E., Hope, J.M., Des Marais, D.J., 1999, Geochimica et Cosmochimica Acta, Vol. 63, No. 1, pp. 79-93; [4] Hanson, R.S., and Hanson, T.E., 1996, Microbiological Reviews, Vol. 60 no. 2; [5] Talbot, H.M., Farrimond, P., 2007, Organic Geochemistry, Vol. 38, pp. 1212-1225; [6] Rohmer, M., Bisseret, P., Neunlist, S., 1992, in Biological Markers in Sediments and Petroleum, pp. 1-17, Prentice Hall, London; [7] Post, W..M., Emanuel, W.R., Xinke, P.J., Strangenberger, A.G., 1982, Nature 298, 156; [8] Dedysh, S.N., Berestovskaya, Y.Y., Vasylieva, L.V., Belova, S.E., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Liesack, W. and Zavarzin, G.A., 2004, Int. Journ. of Syst. and Evol. Microbiology 54, pp. 151-156