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FEMS Microbiology Ecology 54 (2005) 339–350

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Methane cycling in lake sediments and its influence on chironomid larval $\delta^{13}C$

Gundula Eller [a,*], Peter Deines [a], Jonathan Grey [a,1], Hans-Hermann Richnow [b], Martin Krüger [c,2]

[a] *Max Planck Institute for Limnology, August-Thienemann-Strasse 2, 24306 Plön, Germany*
[b] *UFZ Leipzig-Halle GmbH, Permoserstrasse 15, 04318 Leipzig, Germany*
[c] *Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany*

Received 25 January 2005; received in revised form 19 April 2005; accepted 20 April 2005

First published online 23 May 2005

Abstract

Stable carbon isotope analysis of chironomid larvae gave rise to the hypothesis that methane-oxidizing bacteria can provide an important food source for higher trophic levels in lakes. To investigate the importance of the methane cycle for the larval stable carbon signatures, isotope analysis and microbiological and biogeochemical investigations were combined. The study was based on comparison of a dimictic lake (Holzsee) and a polymictic, shallow lake (Großer Binnensee), both located in northern Germany. Both lakes are inhabited by *Chironomus plumosus* larvae, which exhibited a stronger ^{13}C-depletion in Holzsee than in Großer Binnensee, indicating a greater contribution of methane–carbon in the former. Indeed, the processes involved in the microbial methane cycle were found to be more active in Holzsee, showing higher potential methane production and methane oxidation rates. Consistently, cell numbers of methane-oxidizing bacteria were with $0.5 - 1.7 \times 10^6$ cells g_{dw}^{-1} about one order of magnitude higher in Holzsee than in Großer Binnensee. Molecular analysis of the microbial community structure revealed no differences in the methanotrophic community between the two lakes, with a clear dominance of type I methanotrophs. The methanogenic population seemed to be adapted to the prevailing substrate in the respective lake (H_2/CO_2 in Holzsee and acetate in Großer Binnensee), even though differences were minor.

In conclusion, the stronger larval ^{13}C-depletion in Holzsee was not reflected in differences in the microbial community structure, but in the activity and size of the methanogenic and methanotrophic populations in the lake sediment.

Keywords: Carbon cycle; Carbon stable isotopes; Methanogenesis; Archaea; Methanotroph; Chironomid larvae

1. Introduction

Microbial methane production and oxidation are important processes in the mineralization of organic material. They are controlling the sedimentary methane cycle, relevant for the emission of the greenhouse gas methane to the atmosphere. A recent survey on methane emissions from lakes showed that lakes contribute 6–16% of the global natural methane emissions [1]. Besides its importance in the carbon export from lakes, methane oxidation (MO) leads to the build up of microbial biomass, which can be used as an internal carbon source at the basis of the trophic system in the lake.

In metabolic steps along the food web, the preferred reaction of ^{12}C carbon leads to an enrichment of the

* Corresponding author. Tel.: +49 0 4522 763 244; fax: +49 0 4522 763 310.
E-mail address: eller@mpil-ploen.mpg.de (G. Eller).
[1] Current address: School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK.
[2] Current address: Geomicrobiology, Federal Institute for Geosciences and Resources, Stilleweg 2, 30655 Hannover, Germany.

0168-6496/$22.00
doi:10.1016/j.femsec.2005.04.006

lighter isotope ^{12}C instead of ^{13}C in organic compounds, enabling food web studies by stable carbon isotope analysis. The carbon isotope fractionation of methanogenic archaea is pronounced and leads to a distinct, very depleted signature of the methane produced, with $\delta^{13}C$ signatures as low as $-50‰$ to $-110‰$ [2]. The distinct carbon signature of biogenic methane can be used as a tracer for the recycling of methane–carbon into the food web of lakes. Indeed, recent food web studies based on stable carbon isotope analyses of chironomid larvae revealed a possible link between the microbial methane loop in sediments and higher trophic levels in aquatic systems [3–6]. The ^{13}C-depletion found for the larval biomass compared to mean $\delta^{13}C$ values of sediment and particulate organic matter led to the hypothesis that chironomid larvae assimilated a dietary food source based on methane-oxidizing bacteria (MOB), which had converted isotopically light biogenic methane into biomass. A comparison of larval $\delta^{13}C$ and corresponding lake characteristics supported this hypothesis [4].

In this study, we investigated the methane cycle and the isotopic signature of chironomid larvae in parallel, to get a more detailed picture of their mutual interaction. For this purpose, we chose two eutrophic lakes with contrasting morphology: Großer Binnensee (GB), a shallow, well-mixed (polymictic) lake and Holzsee (Hz), a summer stratified (dimictic) lake with regularly occuring anoxia in the hypolimnion, both located in northern Germany. We hypothesized that methane is produced in the sediments of both lakes, but with higher rates in the dimictic lake Hz, fuelling a more active MO and consequently resulting in higher cell numbers and more biomass of MOB in the sediment. The higher availability of MOB biomass as a food source for chironomid larvae leads to a more pronounced ^{13}C-depletion of the larvae in the dimictic lake. Differences in larval $\delta^{13}C$ between lakes, on the other hand, could also be dependent on differences in the methane $\delta^{13}C$ of these lakes, which is strongly influenced by the metabolic pathway used for methane production [2]. The fractionation between methane and the biomass of MOB depends on the prevailing family of MOB, resulting in no fractionation for type II MOB biomass compared to the signature of methane if methane is limiting, and a fractionation of $-13‰$ to $-30‰$ for type I MOB [7,8]. Besides the quantitative aspect of the interaction between methane turnover and larval $\delta^{13}C$, the community structure of methanogens and methanotrophs is important to estimate the isotopic signature of the methane-based food source. Therefore, possible qualitative differences in the methanogenic and methanotrophic community were investigated by denaturing gradient gel electrophoresis (DGGE) as fingerprinting method.

2. Materials and methods

2.1. Sampling sites

The two lakes investigated, GB and Hz, are located in the Holsteiner Lake District, northern Germany (54° 19′ 40″ N, 10° 37′ 30″ E for GB and 54° 09′ 36″ N, 10° 11′ 04″ E for Hz). Their most important characteristics are summarized in Table 1. Hz is a dimictic lake with regular oxygen depletion in the hypolimnion throughout the summer stratification period. GB is shallow (mean water depth 2 m) and polymictic. Sediment and chironomid larvae were sampled in August 2003 at a water depth of 6 m in Hz and 3 m in GB. Oxygen and temperature profiles were measured with a WTW oxygen probe EOT 190 (Oximeter Oxi 191, WTW, Germany).

2.2. Preparation of chironomid larvae and sediment samples for stable carbon isotope analysis

Fourth instar *Chironomus plumosus* larvae were sieved in situ from sediments collected using an Ekman grab. The larvae were left overnight in filtered tap water (0.2 μm) at ambient temperature for gut clearance. Faecal material was removed to prevent coprophagy and fixed with *para*-formaldehyde for fluorescence in situ hybridization (FISH). Individual larvae were oven-dried at 60 °C, homogenized and stored for subsequent stable isotope analysis by continuous flow isotope ratio mass spectrometry as described by Grey et al. [3]. Sediments were acidified (0.5 M HCl) for 24 h to remove inorganic carbon according to Midwood and Boutton [9], and prepared and analyzed as for chironomids. The isotopic ratios were expressed in the delta notation: $\delta^{13}C = 10^3(R_{sa}/R_{st} - 1)$ with $R = {}^{13}C/{}^{12}C$ of sample (sa) and standard (st), respectively [3,10].

Table 1
Characteristics[a] of the dimictic Holzsee and the polymictic Großer Binnensee

Lake	Surface area (km^2)	Maximum depth (m)	Mean depth (m)	DOC (mg C l^{-1})	N:P	pH	O_2 (mg l^{-1})	T (°C)
Großer Binnensee	4.8	3.0	1.9	8.2	31	8.0	10.6/9.2	23.9/23.8
Holzsee	0.2	6.9	3.7	5.0	24	7.9	9.5/0.1	25.6/12.8

[a] Data represent mean values for the years 1991–2000 [59], except for oxygen concentrations and temperatures, which were measured at the water surface (left) and above the sediment surface (right) during the current study.