TITLE: ACTIVE ANAEROBIC OXIDATION OF METHANE IN AMAZONIAN FLOODPLAINS: A POSSIBLE LINK BETWEEN THE METHANE AND NITROGEN CYCLES

AUTHORS: MONTEIRO, G. G. T. N. [1]; BARROS, D. J.[2]; VENTURINI, A.M.[3]; NEU, V.[4]; TSAI, S. M.[1]; NAVARRETE, A. A.[5]

INSTITUTION: 1. Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil; 2. Federal University of Tocantins; 3. Princeton Institute for International and Regional Studies, Princeton University, Princeton, NJ, USA; 4. Federal Rural University of the Amazon; 5. University Brazil – Graduate Program in Environmental Sciences.

ABSTRACT:

Methane (CH_4) is a greenhouse gas with approximately 34 times the potential of carbon dioxide (CO_2). The Amazonian floodplains are an important natural source or sink of CH_4 in the tropics, which depends on the balance between CH_4 production (methanogenesis) and consumption (methanotrophy) by specific microbial communities. Methanotrophy can occur under anaerobic environments coupled with the reduction of sulfate (SO_4^{-2}) nitrate (NO_3^-), nitrite (NO_2^-), and other metals. Thus, through microbial activity, most of the CH_4 produced during the flooding period of the Amazonian floodplains can be consumed before it diffuses into the atmosphere. To better understand the anaerobic methanotrophic microorganisms, we analyzed the anaerobic methane-oxidizing (ANME) microbial community of different Amazonian floodplain soils through 16S rRNA amplicon sequencing. Total RNA was isolated from 36 soil samples (three floodplains × two land uses × two seasonal periods × three sampling points) using the RNeasy PowerSoil Total RNA Kit. Complementary DNA (cDNA) was synthesized using a QuantiNova Reverse Transcription Kit, and amplicon libraries were prepared using the cDNA as the template in the amplification reactions with specific archaeal and bacterial primers for the 16S rRNA genes. The final library was sequenced on a MiSeq Personal Sequencing System. All 16S rRNA gene sequence reads were processed and analyzed using QIIME2 v.3.5.3. with the SILVA database. Statistical analyses were carried out using R in the RStudio environment. The ANME community in the floodplain soils was mostly composed of two microbial groups related to the anaerobic methane oxidizers *'Candidatus Methylomirabilis oxifera'* and *'Candidatus Methanoperedens nitroreducens'*. In CH_4-rich environments, these methanotrophs are capable of establishing a consortium and oxidizing CH_4 while reducing NO_3^- to NO_2^- and subsequently coupling NO_2^- to denitrification, effectively linking the carbon to the nitrogen cycle. The establishment and the putative role of this consortium in the methanotrophy is also apparently modulated by the flood pulse of Amazonian rivers and occurs mainly in the rainy season, which highlights the importance of the hydrological cycle in the modulation of the CH_4 cycle on the Amazonian floodplains.

Keywords: Anaerobic methane oxidation, anaerobic methane-oxidizing bacteria and archaea, amplicon sequencing, carbon and nitrogen cycle, microbial ecology.

Acknowledgments: We thank Marília de Souza Bento and Leonardo Machado Pitombo for their overall support, Ivanildo L. S. Gaia, Glauber Altrão Carvalho, and Domickson Costa for their support during fieldwork. We also thank the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brasil (FAPESP) for the grant that made this study possible (2016/16687-3).