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Subterranean microbial oxidation of atmospheric methane in cavernous tropical karst

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ABSTRACT

Subterranean methanotrophy is a potentially important but overlooked sink for the atmospheric greenhouse gas methane (CH_4). This study documents a microbial CH_4 sink in tropical subterranean karst cavities in Vietnam's northern karst province where porosity, steep topography, and scarce soil and vegetation cover foster the exchange of subterranean air with the atmosphere. Our data are based on (i) surveys of CH_4, carbon dioxide, and radon concentrations in the air of 11 caves, (ii) *in situ* mesocosm experiments in caves, as well as (iii) laboratory mesocosm measurements using sediment and rock from caves. The extent of CH_4 depletion in cave air depends on the ventilation rate and the availability of moisture to provide a habitat for CH_4-oxidizing bacteria, both of which are seasonally variable in northern Vietnam and in part depend on monsoonal activity. Mesocosm experiments using fresh *versus* sterilized rock and sediment confirmed the role of microbial methanotrophy towards uptake of CH_4 from cave air. Our results also suggest that within-cave heterogeneity of environmental variables like salinity may affect rates of CH_4 oxidation. We conservatively estimate that 150,000 metric tons of atmospheric CH_4 are microbially oxidized annually in the ~29,000 km^2 of Vietnamese tropical karst, which would compensate for ~7% of Vietnam's agricultural CH_4 emissions from rice farming and livestock. Future studies estimating the global fluxes of the atmospheric greenhouse gas CH_4 should consider subterranean karst as a potentially important CH_4 sink.

1. Introduction

Atmospheric methane (CH_4) is a potent greenhouse gas with globally and rapidly rising concentrations mainly due to anthropogenic activities (Etminan et al., 2016; Ciais et al., 2013; IPCC, 2013; U.S. EPA, 2010). Credible forecasting of global warming by climate models mandates knowledge of sources and sinks of atmospheric CH_4 (Xu et al., 2016). The two traditionally recognized major sinks for CH_4 are oxidation by (i) the hydroxyl radical in the upper atmosphere, and (ii) soil CH_4-oxidizing bacteria (MOB) found in soil and sediments (Naik et al., 2013; Dlugokencky et al., 2011). MOB habitats can also be found in other subterranean oxic environments with access to CH_4. Earth's continental surface includes ca. 10 to 20% of karst landforms (see p. 5 in Ford and Williams, 2007; Palmer, 1991) where caves and abundant smaller-size cavities and fractures provide abundant surface area and suitable habitat for MOB. The interconnected subterranean cavities in karst not only serve as drainage networks, but also provide conduits for air exchange with the atmosphere containing ~1.8 parts per million by volume (ppmv) CH_4 (Atkinson et al., 1983; Gregorič et al., 2014).

Several studies in temperate European, North American, and Australian regions have documented cases of CH_4 depletion in cave air relative to outside air (e.g., Waring et al., 2009; Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016; Webster et al., 2016), but studies from tropical karst have been lacking. In contrast to karst in temperate climates, the exchange of subterranean air with the atmosphere in northern Vietnam's karst province and associated subterranean methanotrophy are fostered by the scarcity of soil and vegetation cover on the surface, a steep topography of abundant limestone outcrops, warm temperatures, and almost year-round availability of liquid water on many rock surfaces and in sediment. The fate of CH_4 in cave air is not only important from the perspective of a greenhouse gas sink, but CH_4 is also able to provide a carbon and energy source for

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heterotrophic subterranean biota (Jones and Macalady, 2016).

An assessment of CH_4 abundances and sinks in subterranean karst air is most easily accomplished in accessible caves, although the same biochemical and geochemical processes likely occur in smaller-size cavities that connect to the atmosphere. The seasonal dynamics and heterogeneity of trace gas compositions in cave air strongly depend on the rate and direction of ventilation, especially if a cave has multiple large openings for air access. This study used hydrogen-filled, neutrally buoyant balloons for accurate measurements of wind speed in cave passages. The ventilation of caves can be constrained by understanding the flux of carbon dioxide (CO_2) and radon in cave air. For example, a longer residence time of air in caves tends to decrease the CH_4 concentration, but often leads to accumulation of carbon dioxide and radon (e.g., Atkinson et al., 1983; Kowalczk and Froelich, 2010; Gregorič et al., 2014). None of the studied caves in northern Vietnam exhibited a sustained and strongly elevated radon concentration to plausibly explain low CH_4 concentrations *via* abiotic oxidation that is triggered by radioactivity in cave air (Fernandez-Cortes et al., 2015).

Few data have been published about rates of CH_4 oxidation in cave air. This study's data on rates of MOB activity from 11 caves add to previously available limited evidence from two caves on Cát Bà Island in coastal northern Vietnam (Lennon et al., 2017). In spite of substantial spatial and temporal variability of CH_4 uptake in cave environments, the available matrix of observations in, and CH_4 profiles along caves encouraged us to synoptically arrive at an overall rough estimate of CH_4 biodegradation in Vietnamese karst. Field and laboratory data on CH_4 depletion in contact with karst rock and sediment suggest that subterranean CH_4 oxidation in tropical karst represents a globally significant sink for atmospheric CH_4.

2. Materials and methods

2.1. Characterization of field sites

Karst occupies ~9% (i.e. ~29,000 km^2) of Vietnam's land surface and occurs in central and northern Vietnam (Trần and Nguyễn, 1986; Trần, 2005). The ~22,000 km^2 of northern Vietnamese karst represent ~19% of the northern region (Trần, 2005) within a tropical humid belt featuring typical tropical karst landforms, such as dolines, deeply incised valleys, and caves. The northern karst outcrops comprise mostly carbonates that are intercalated with minor terrigenous and/or marine siliceous members (Trần and Nguyễn, 1986; Do, 1998; Tran et al., 2013) and span from Early Cambrian to late Mesozoic ages. Regional tectonic activities variably caused thickening, fragmentation and dismemberment of rock units. It has been proposed that the development of the unique karst landscape in northern Vietnam resulted from a combination of deposition, deformation (Tran et al., 2013), and recent sedimentary and weathering processes in a tropical monsoon climate (Dang et al., 2009).

We conducted field work in 11 caves in May and November/December 2015 in (i) the mountainous karst in Vietnam's most northern Hà Giang province (Masschelein et al., 2007) and (ii) in low-altitude, coastal karst on Cát Bà Island (Fig. 1; Table 1). A main part of the northern Vietnamese mountainous karst area is the *Đồng Văn Karst Plateau Geopark* where some of the visited caves are located (GGN, 2010).

The monthly mean temperature and precipitation profiles for the city of Hà Giang in Hà Giang Province and for Cát Bà Island are shown as hythergraphs (Fig. 2; National Hydro-Meteorological Service of Vietnam, 2012). The monsoonal pattern of peak precipitation in summer with contemporaneously elevated temperatures is apparent for both regions, although Hà Giang tends to be slightly cooler than coastal areas and its precipitation is essentially symmetrically distributed around July as the wettest month, whereas precipitation on Cát Bà Island is left-skewed with more precipitation occurring in late summer. Seasonal monsoon rain provides for groundwater flow, whereas surface water flow is rare due to highly permeable karst rock with generally sparse soil cover.

2.2. In situ gas analyses in caves

Gas measurements in caves were performed in readily accessible locations where instruments could be positioned on level ground. Operating personnel stepped aside to avoid artifacts from human breath. When the entrances and passages of some caves proved to be too hazardous for transporting sensitive instruments, we organized teams to capture 200-L volumes of cave air in large plastic bags. The sampling of air avoided the influence of human breath. The bags were then rapidly carried to safe locations where gas samples were transferred into instruments with short segments of Tygon® plastic tubing. Concentrations of CH_4 and CO_2 in cave air were measured in May and Nov/Dec 2015 with a portable Gasmet DX-4030 FTIR analyzer (*Gasmet Technologies Oy*, Finland) that was calibrated daily with ultrapure nitrogen gas. The precision of measured gas concentrations is ± 0.3 ppmv for CH_4 and ± 30 ppmv for CO_2 (± 95% confidence intervals). Additional CH_4 and CO_2 measurements were performed with a SARAD® RTM 2200 instrument (*SARAD®*, Dresden, Germany, with an Axetris® laser OEM Module LGC F200 CH_4 detector, *Axetris® AG*, Switzerland) that had been calibrated against the Gasmet DX-4030 FTIR analyzer. With detailed data from Nà Luông, Rồng, and Pải Lủng caves, we were able to map CH_4 and CO_2 concentrations by gridding unevenly distributed data with adjusted-tension continuous curvature splines using the surface module of Generic Mapping Tools (Wessel et al., 2013) with a tension factor 0.25, convergence limit 0.01, and 1.5 m distance between estimated points.

Radiation from the decay of radon (^{222}Rn) in caves was α-spectroscopically quantified with the portable SARAD® RTM 2200 instrument. We did not encounter high CO_2 concentrations that would have limited the precision of the SARAD® RTM 2200 (Shahrokhi et al., 2015).

Wind speed in caves was measured by the motion of free-floating hydrogen-filled latex balloons. Hydrogen gas was generated on-site in a 1-L bottle that was half filled with water and received ~3 g of calcium metal turnings wrapped in tissue paper. A deflated latex balloon was connected to the mouth of the bottle and became inflated as elemental hydrogen gas developed pressure. Inflated balloons were tied with short strings and ballasted with adhesive tape to neutral buoyancy before being released in cave passages.

2.3. Mesocosm experiments

We performed mesocosm experiments to test for methanotrophy in a cave (using cave rocks) and in the laboratory in Hanoi (using 10 sediments and 1 rock sample from 5 different caves; Table 2). Rocks weighing from 0.1 to 1 kg were collected in Minh Châu cave, whereas fine-grained, clay-rich sediments from cave floors were shoveled into polyethylene bags. Sediments were not allowed to lose moisture prior to their use in mesocosm experiments in the laboratory in Hanoi within a few days after transport. In contrast to our earlier mesocosm experiments in Minh Châu cave that had used single-layer polyethylene bags (Lennon et al., 2017), in this study we attempted to minimize diffusive loss of CH_4 through the 0.0475 mm thick polyethylene bags by nesting one bag within another (i.e. 'double-bags'). We conducted *in situ* double-bag control experiments in Minh Châu cave by spiking 200 L of air with a few milliliters of CH_4 from biogas (~90 vol% CH_4) and quantifying the diffusive loss of CH_4 over ~13 to 14 h. Biogas was obtained from a farmer's fermentation plant that was operated with organic farm waste near Hanoi. The 'sweet' smell of CH_4-rich biogas proved the absence of hydrogen sulfide. In contrast to industrially generated CH_4, biogas does not contain acetylene, which can deactivate methanotrophy (Pham et al., 2015). Some mesocosm experiments were conducted in Minh Châu cave where overnight deployment of polyethylene double-bags with local rocks tested for patterns of *in situ*