Soil Biology and Biochemistry 119 (2018) 90–100

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Denitrifying anaerobic methane oxidizing in global upland soil: Sporadic and non-continuous distribution with low influence

Guibing Zhu[a,d,*], Mengzi Wang[a], Yixiao Li[a,b], Yu Wang[c], Hexin Fei[a], Shanyun Wang[a]

[a] Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Shandong Provincial Key Lab of Environmental Engineering, Qingdao University of Technology, Qingdao 266033, China
[c] Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China
[d] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Damo
Methane oxidation
Activity
Upland soil
Global scale

ABSTRACT

Both the distribution and contribution of denitrifying anaerobic methane oxidation (damo) have been extensively reported in enrichment culture and aquatic ecosystems; yet, the role of damo in upland soil remains unclear. In the present study, this role of damo bacteria in global upland soils was investigated via isotope tracing technique, quantitative PCR assay, and clone library construction, targeting both 16S rRNA and functional *pmo*A genes. Using samples from the surface (0–20 cm, $n = 148$) and deep soil profiles (from 0 to 70–6300 cm, $n = 160$), the damo bacterial distribution seemed to be sporadic in surface soils and non-continuous in soil profiles. The moisture content of the upland soils seems to be the key factor to influence damo occurrences. Among soil profiles with higher moisture content, the highest damo bacterial abundance of 4.62×10^6 copies g^{-1} soil was detected at 180–200 cm depth in Xingtai, China. Both a shift of damo bacterial community structure was observed with depth as well as strong temporal heterogeneity within surface soils. Damo activity (0.18 nmol C g^{-1} h^{-1}) was only detected in the summer surface soil (0–20 cm) of Xingtai. The global distribution of damo in upland soils indicates that upland soils are not favorable environments for the occurrence of damo processes, and the potential role of damo in upland soils might have a limited contribution to the global CH_4 cycle.

1. Introduction

Methane (CH_4) is a greenhouse gas, responsible for ca. 20% of the global warming since the industrial age (Knittel and Boetius, 2009). The global warming potential of CH_4 is about 28 times higher than that of CO_2 within a 100-year time frame (Grubler, 2014). CH_4 was previously assumed to be inert under anoxic conditions (Strous and Jetten, 2004). However, the discovery of denitrifying anaerobic methane oxidation (damo), where CH_4 is anaerobically oxidized, using nitrite as electron acceptor (Raghoebarsing et al., 2006; Ettwig et al., 2010; Haroon et al., 2013), has fundamentally changed our understanding of the biogeochemical CH_4 cycle.

Damo was first demonstrated in an enrichment culture from freshwater sediments (Raghoebarsing et al., 2006; Ettwig et al., 2008) and the microorganism that mediated this reaction was named *Candidatus Methylomirabilis oxyfera* (*M. oxyfera*) belonging to the NC10 phylum (Ettwig et al., 2009, 2010). Subsequently, damo bacteria were successfully enriched in wastewater treatment plants (Luesken et al., 2011a) and paddy soils (Hatamoto et al., 2014). Molecular or genetic surveys showed extensive presence of damo bacteria, e.g. in freshwater lake sediments (Deutzmann and Schink, 2011; Kojima et al., 2012), river sediments (Shen et al., 2014; Zhu et al., 2015), estuarine sediments (Chen et al., 2014), marine sediments (Chen et al., 2015), and wetland ecosystems (Shen et al., 2014; Wang et al., 2016). In aquatic ecosystems, the damo process was identified as an important component of the global carbon cycle and to act as a previously overlooked microbial CH_4 sink (Hu et al., 2014). However, most calculations of the global carbon budget have not taken damo processes into account (Hu et al., 2014). It is therefore necessary to investigate the contribution and role of damo processes in agricultural soil systems to enable more accurate nitrogen and carbon cycle models. Compared to extensive studies in wetlands and paddy soil ecosystems (Zhu et al., 2010; Wang et al., 2012a; Hu et al., 2014; Zhou et al., 2014; Shen et al., 2015; Zhu et al., 2015), the occurrence, activity, and contribution of damo in upland soil remain poorly investigated to date.

Wetland ecosystems have been deemed as CH_4 sources for a long

* Corresponding author. Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
E-mail address: gbzhu@rcees.ac.cn (G. Zhu).

https://doi.org/10.1016/j.soilbio.2018.01.011
Received 11 July 2017; Received in revised form 2 January 2018; Accepted 16 January 2018

time (Grubler, 2014). Various studies about the contribution and activities of damo in wetland and paddy soil ecosystems indicated that the damo process occurred in environments with high CH_4 concentration and oxygen-limited ecosystems (Zhu et al., 2010; Wang et al., 2012a; Hu et al., 2014; Zhou et al., 2014; Shen et al., 2015; Zhu et al., 2015; Bhattacharjee et al., 2016). Wetlands and paddy soils become saturated during flooding; hence, anoxic soil layers provide a likely habitat for damo bacteria. In contrast to paddy soil, which is a significant source of CH_4, the upland soil was identified to be a sink of CH_4 via aerobic methane oxidation (Barone et al., 2011; Sun et al., 2016). Various studies have also confirmed that CH_4 release from upland soils is low (Knief et al., 2003; Sun et al., 2016). Moreover, oxygen transfer in the plant rhizosphere provides an aerobic rather than anoxic environment in the unsaturated soil at the surface of upland soil, which is not conducive for NO_2^- accumulation (Hui et al., 2017). Therefore, we hypothesized that upland soil was not a preferred habitat for damo bacteria, and that the damo process would not be actively involved in CH_4 oxidation in upland soils.

The objective of this study was to investigate the occurrence, distribution, and activity of the damo process in upland soils. Soil samples were collected from various countries and analyzed using molecular, and ^{13}C and ^{15}N based isotope-tracing techniques. In addition, the interspecies relationships among damo bacteria, denitrifiers, and nitrifiers, which are affecting damo by either providing nitrite or competing for nitrite, were analyzed to investigate the role of damo in upland soils.

2. Materials and methods

2.1. Site description and sampling

A total of 148 surface upland soils (0–20 cm) were collected from Asia, North America, South America, Europe, Africa, and Australia (Fig. 1); nine of these sampling sites were selected to further investigate the vertical distribution of damo bacteria. Characteristics and environmental description (e.g. farmland, grassland, or forestland) of the collected soil samples are summarized in Table 1. For each surface sampling site, three samples were collected parallel to each other from three plots (10 m × 10 m). For soil profile samples, three soil pits were dug also from a 10 m × 10 m × 10 m field at each sampling site. Samples separately collected in June and December from Xingtai (Hebei Province, China) were examined last. Core samples were collected in each pit and sliced at specific intervals.

Soil samples were collected using a 7 cm diameter auger, were stored in sterile plastic bags, sealed, and transported to the laboratory while being stored on ice. Subsequently, three parallel fresh soil samples of each depth from the same sampling site were mixed to form one composite sample, and visible roots and residues were removed prior to homogenizing the soil fraction of each subsample. A portion of the fresh subsample was immediately incubated after arrival to determine damo activity and related N-cycle processes, according to ^{13}C- or ^{15}N-isotopic tracing technologies, respectively (McIlvin and Altabet, 2005; Füssel et al., 2012; Hu et al., 2014; Shen et al., 2015). A further portion was air-dried and sieved through a 2.0 mm mesh for chemical component analysis. A further small fraction of the subsamples were freeze-dried at −50 °C and stored at −80 °C for DNA extraction and subsequent molecular analysis. The details for each analysis are presented in the chapters below.

2.2. Analytical procedures of soil properties

Ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) were extracted via 2 M KCl solution, shaken for 1.5 h at 150 rpm and then determined via flow injection analysis (San++, SKALAR, the Netherlands). The total inorganic nitrogen (TIN) content was calculated via the sum of ammonium, nitrite, and nitrate. The pH was analyzed via DELTA 320 pH Analyzer (Mettler Toledo, USA) after shaking soil water (1:5 wt/vol) suspension for 30 min. The moisture contents of upland soils were measured by oven-drying 2 g fresh soil at 108 °C until a constant weight was obtained. The total organic matter (TOM) was measured via LOI_{550} (loss on ignition at 550 °C) (Bao, 2000). The methane concentration profiles in vertical upland soils were determined according to a previously published method (Kojima et al., 2012) when the soil moisture content was above 25%. Briefly, approximately 2 g soil was thoroughly mixed with 0.4 g NaCl and 1 mL distilled water in a vial. The obtained slurry was heated at 60 °C for 30 min to accelerate the methane release. The concentration of the methane released into the head space was measured using a gas chromatograph (Shimadzu, Japan). Triplicates were run for each measurement.

2.3. DNA extraction, PCR, cloning, and sequencing

DNA was extracted from approximately 0.33 g freeze-dried soil using a FastDNA SPIN Kit for Soil (Bio 101, USA) according to the manufacturer's protocol. The quality of DNA was evaluated on 1% (*w/v*) agarose gel. PCR for the damo bacterial *pmo*A gene was performed as previously described (Luesken et al., 2011b; Zhu et al., 2015). Primers and thermal profiles of the PCR are shown in Table S1. The PCR products were cloned with pGEM-T Easy Vector (Promega, USA) and *Escherichia coli* JM109 competent cells following the manufacturer's

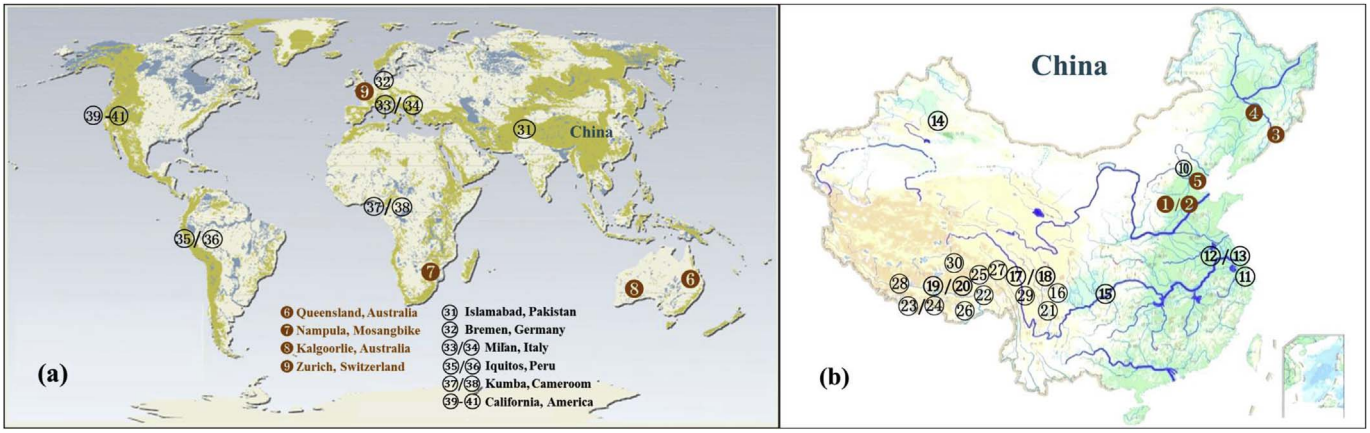

Fig. 1. The distribution of the sampling sites where surface upland soil samples (in black words) and upland soil cores samples (in red words) were collected. The samplings sites were numbered with the sequence that appeared in Table 1. (a) The world map was reproduced with permission from the Millennium Ecosystem Assessment 2005 (http://www.MAweb.org), UNEP; (b): The map of China was cited from National Earth System Science Data Sharing Infrastructure, National Science & Technology Infrastructure of China (http://www.geodata.cn). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)