Electron shuttling mediated by humic substances fuels anaerobic methane oxidation and carbon burial in wetland sediments

Edgardo I. Valenzuela [a], Karen A. Avendaño [b], Nagamani Balagurusamy [b], Sonia Arriaga [a], Cesar Nieto-Delgado [a], Frederic Thalasso [c], Francisco J. Cervantes [a,*]

[a] *División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, Mexico*
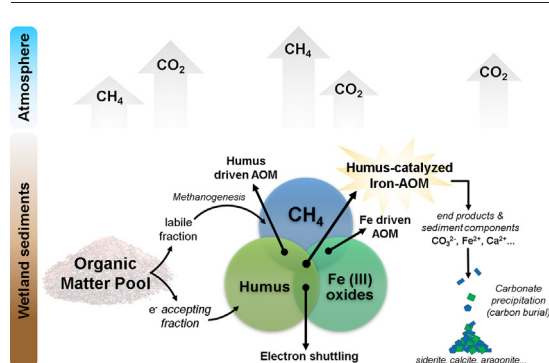[b] *Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Torreón, Coahuila, Mexico*
[c] *Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav-IPN), Av. IPN 2508, Mexico City, Mexico*

HIGHLIGHTS

- Elusive mechanisms for AOM in a tropical wetland were explored.
- Humus promoted net AOM with a poorly-reactive ferric oxide as electron sink.
- Humus-driven electron shuttling enhanced carbon sequestration as inert minerals.
- Environmental significance of humus electron shuttling in AOM is discussed.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Key pathways for the anaerobic oxidation of methane (AOM) have remained elusive, particularly in organic rich ecosystems. In this work, the occurrence of AOM driven by humus-catalyzed dissimilatory iron reduction was investigated in sediments from a coastal mangrove swamp. Anoxic sediment incubations supplied with both *goethite* (α-FeOOH) and *leonardite* (humic substances (HS)) displayed an average AOM rate of $10.7 \pm 0.8\ \mu mol\ CH_4\ cm^{-3}\ day^{-1}$, which was 7 and 3 times faster than that measured in incubations containing only goethite or HS, respectively. Additional incubations performed with ^{13}C-methane displayed *Pahokee Peat* HS-mediated carbonate precipitation linked to $^{13}CH_4$ oxidation and *ferrihydrite* reduction (~1.3 µmol carbonate $cm^{-3}\ day^{-1}$). These results highlight the role of HS on mitigating greenhouse gases released from wetlands, not only by mediating the AOM process, but also by enhancing carbon sequestration as inert minerals (*calcite*, *aragonite* and *siderite*).

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1. Introduction

Wetlands are complex ecosystems characterized by the flooding of soils and are important in global carbon dynamics because of their large soil carbon pools and potential for carbon sequestration in peat formation, sediment deposition, and plant biomass. These ecosystems, commonly classified as bogs, marshes and swamps, represent the largest biogenic source of greenhouse gases, particularly methane (CH_4), and growing evidence suggests that these emissions are climate sensitive; thus, future CH_4 wetland budgeting will be tightly controlled by global warming (Arneth et al., 2010; Bridgham et al., 2006; Davidson

and Janssens, 2006). The net amount of CH_4 released to the atmosphere from wetlands is estimated to be one third of the global CH_4 budget (~164 Tg yr^{-1}) (Bridgham et al., 2013), and is the result of the balance between methanogenesis (microbial generation of CH_4), and methanotrophy (biological oxidation of CH_4) (Kirschke et al., 2013), with the latter process being promoted by the reduction of molecular oxygen (Trotsenko and Murrell, 2008) or an alternative electron acceptor (sulfate, nitrate/nitrite, oxidized metals, humic material) (Cui et al., 2015; Valenzuela et al., 2017). Due to limited oxygen availability induced by flooding conditions, anaerobic oxidation of methane (AOM) constitutes a key CH_4 sink in wetlands with the potential to exert outstanding impact on global biogeochemical carbon cycling (Segarra et al., 2015). After decades of elusiveness, AOM in wetlands has been linked to the reduction of sulfate (Segarra et al., 2013, 2015), nitrate/nitrite (Hu et al., 2014; Shen et al., 2014, 2017; Zhu et al., 2012), and recently to the electron-accepting fraction of natural organic matter (NOM), also known as *humus* or *humic substances* (HS) (Valenzuela et al., 2017). HS is the term traditionally employed to denote the recalcitrant fraction of NOM with redox activity (capability to accept or donate electrons) (Lovley et al., 1996, 1999; Stevenson, 1983), which has also been referred to as *organic acids* (Lehmann and Kleber, 2015). Once HS are reduced by microbial activity, they can be chemically reoxidized, either by molecular oxygen (Klüpfel et al., 2014) or oxidized compounds, such as metallic oxides (Lovley et al., 1998; Scott et al., 1998) promoting further oxidation of pollutants. Due to their abundance on Earth and availability in both terrestrial and aquatic environments, the oxidized forms of iron, which at near neutral pH values prevail in the form of crystalline minerals, are the most important metals with the capacity to re-oxidize HS in natural ecosystems (Lovley et al., 1998). To date, iron-dependent AOM has been reported in several aquatic ecosystems including marine sediments (Beal et al., 2009; Egger et al., 2015; Riedinger et al., 2014; Wankel et al., 2012), a polluted aquifer (Amos et al., 2012), and freshwater lakes (Bar-Or et al., 2017; Nordi et al., 2013) by means of isotopic tracing and biogeochemical profiling (He et al., 2018). Nevertheless, the role of HS on this reaction has not been demonstrated despite the fact that electron shuttling reactions (involving both metals and sulfurous compounds) have been previously demonstrated to play key roles in the dynamics of anaerobic degradation of organic molecules (Heitmann et al., 2007; Heitmann and Blodau, 2006; Lovley et al., 1998). The occurrence of *humus-mediated* iron-dependent AOM, firstly hypothesized almost a decade ago (Smemo and Yavitt, 2011), would represent an unrevealed methane sink that could have huge biogeochemical and ecological implications, since the net amount of CH_4 mitigated by ferric iron and HS in organotrophic ecosystems may be much higher than previously considered due to the redox interactions involved (Lovley et al., 1998; Martinez et al., 2013; Reed et al., 2017). Until now, it has been estimated that AOM contributes to suppress methane emissions in freshwater wetlands by 200 Tg of methane $year^{-1}$ (Segarra et al., 2015).

Given the recent evidence on the potential role of HS as an electron acceptor in the AOM (Scheller et al., 2016; Valenzuela et al., 2017), we aimed to provide biogeochemical insight into the role of humus-catalyzed reduction of iron oxides in the AOM in wetland sediments. To this end, we studied the addition of two widespread mineral forms of ferric iron, and two sources of external HS on the methanotrophic activity carried out by the biota of a coastal wetland sediment, which previously showed AOM activity linked to the reduction of HS and their analogues (Valenzuela et al., 2017). Three scenarios were considered: 1) a long-term incubation in which depletion of intrinsic electron acceptors allowed substantial rates of net AOM with a highly crystalline iron oxide (e.g. goethite) as terminal electron acceptor, 2) an incubation cycle in which the biogeochemical conditions prevailing in the sampled site were preserved, and 3) an incubation under artificial conditions using destructive sampling to minimize the interference related to the biogeochemical complexity of natural samples. The reduction of different external electron acceptors (two sources of HS and two iron oxides of different reactivity) was correlated to AOM both as $^{13}CO_2$ enrichment and as net rates of ^{12}C and ^{13}C-methane consumption.

2. Materials and methods

2.1. Wetland description and sediment sampling

The *Sisal* wetland is a mangrove swamp located within the ports of Celestun and Sisal in the Yucatán Peninsula, southeastern Mexico (21°09′26″N, 90°03′09″W). This coastal zone has a semi-arid climate and presents a high degree of karstification of sediments, which are mainly composed of tertiary carbonates subject to constant dissolution. Due to intermittent saltwater inputs from the ocean (which reach 40 km inland) (Batllori-Sampedro et al., 1999), this water reservoir possesses significant levels of salinity, which can vary seasonally depending on the rainfall regime. Wetland sediment samples were collected in January 2016, the depth of the sampled cores being 15 cm. Sediment cores were harvested under a water column of approximately 70 cm. Water samples from the top of the sampling point were also collected. All samples were sealed in hermetic flasks and maintained in ice until their arrival to the laboratory where they were then stored at 4 °C in a dark room. Before performing the incubation assays, sediment and water samples were chemically characterized. The characteristics of sediment and water samples are provided in Supplementary Material (SM, Table S1).

2.2. Microcosms set-up

2.2.1. Long-term incubations

2.2.1.1. Inoculation and first incubation cycle. A wetland sediment core was homogenized inside of an anaerobic chamber (COY 14500; atmosphere composed of N_2/H_2, 95%/5%, v/v) and 2 g of wetland sediment were inoculated into 120 mL serological flasks. Subsequently, 30 mL of anoxic artificial basal medium with the following composition (in g L^{-1}): NH_4Cl (0.3), K_2HPO_4 (0.2), $MgCl_2 \cdot 6H_2O$ (0.03), $CaCl_2$ (0.1), NaCl (3), and 1 mL L^{-1} of a trace metals solution previously described (Valenzuela et al., 2017), were provided as liquid medium. In order to provide the different experimental conditions, the artificial basal medium was enriched with the following electron acceptors in different experimental treatments: 5000 mg L^{-1} of bulk leonardite (catalogue number from International Humic Substances Society: BS104L), 50 mmol L^{-1} of chemically synthetized goethite (Cornell and Schwertmann, 2003), a combination of both, or none (unamended sediment control). Six flasks were inoculated under each experimental condition and afterwards they were sealed with air-tight rubber stoppers and aluminum caps and flushed with a mixture of $N_2:CO_2$ (80%/20% [vol/vol]) for 10 min. Before initial headspace measurements and incubation at 28 °C in the dark, three flasks from each experimental treatment were spiked with 30 mL of CH_4 (99% purity; Praxair), reaching a partial pressure of ~0.39 atm (on a 90 mL headspace), while the remaining three were left with a $N_2:CO_2$ atmosphere to serve as *endogenous controls*. Killed controls (amended with the same concentrations of electron acceptors (leonardite and goethite)) were also prepared by autoclaving the sealed microcosms three times at 120 °C for 21 min and then spiked with the same volume of CH_4.

2.2.1.2. Second incubation cycle. After 65 days of incubation in which substantial methanogenesis occurred (during the first 45 days of incubation), a subsequent decrease in methane concentration was observed (net AOM). After this period, microcosms were opened inside the anaerobic chamber, where they were decanted to replace the old liquid medium with freshly prepared basal medium enriched with the same electron acceptors at the following concentrations: 2500 mg L^{-1} of leonardite, 50 mmol L^{-1} of goethite, the combination of both, or none (note that the concentration of leonardite was lowered (50%) with