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Original article

Methane oxidation in response to iron reduction-oxidation metabolism in tropical soils☆

Santosh Ranjan Mohanty [a, *], G.S. Bandeppa [a, b], Garima Dubey [a], Usha Ahirwar [a], Ashok Kumar Patra [a], Kollah Bharati [a]

[a] Indian Institute of Soil Science, Nabibagh, Bhopal, 462038, India
[b] Indian Institute of Rice Research, Rajendranagar, Hyderabad, 500030, India

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ABSTRACT

Experiments were carried out to understand how iron (Fe) reduction-oxidation (IRO) influences CH_4 oxidation in soil. Soil samples (alluvial and vertisol) were induced to undergo microbial Fe reduction and aerobic oxidation consecutively for three cycles simulating natural wetting-drying soil cycle. After each IRO cycle, soils were incubated to determine CH_4 oxidation rate, Fe mineral and methanotrophs abundance. Potential iron reduction rate k (μM Fe^{2+} produced g^{-1} soil d^{-1}) increased from 1.26 to 2.16 in vertisol and 1.95 to 3.05 in alluvial soil. Potential iron oxidation (μM Fe^{2+} oxidized g^{-1} soil d^{-1}) increased from 2.33 to 5.70 in vertisol and 2.43 to 9.58 in alluvial soil. The iron reduction-oxidation significantly ($p < 0.05$) stimulated CH_4 oxidation rate k. The high affinity CH_4 oxidation rate increased from 0.03 to 0.19. Low affinity CH_4 oxidation rate increased from 0.05 to 0.47 in vertisol. A similar effect of IRO on k was observed in alluvial soil. X ray diffraction (XRD) revealed that diffraction intensity of magnetite and goethite decreased over IRO cycle. Real time PCR quantification of methanotrophs (*pmoA* gene) confirmed that IRO cycle stimulated ($p < 0.05$) methanotrophs abundance. The study highlights that iron reduction-oxidation cycles can significantly enhance CH_4 oxidation in tropical soils.

1. Introduction

Iron is the fourth most abundant element and one of the most redox-active metal species found in the earth crust. Iron can form stable minerals in both the divalent and trivalent state depending on geochemical conditions. In natural ecosystem iron reduction-oxidation (IRO) cycle is coupled to cycles of other elements like carbon, nitrogen, phosphorous, and sulfur [1,2]. Iron metabolism is carried out by both chemo-lithotrophic and chemo-organotrophic organisms [3]. Some photoautotrophic bacteria also oxidize Fe^{2+} under anoxic conditions [4]. Iron reduction-oxidation cycles occur in redox transition zone, where Fe^{2+} oxidation is mediated by oxygen or nitrate and Fe^{3+} reduction by anaerobic microorganisms [5,6].

CH_4 is the second important greenhouse gas and its ambient concentration is increasing due to anthropogenic activities [7]. CH_4 affects the earth's atmospheric chemistry and also depletes stratospheric ozone. Therefore, CH_4 oxidation is an important process to regulate global climate change. To curb climate change ambient CH_4 concentration should be lowered and an option could be to enhance oxidation of CH_4 in natural ecosystem. CH_4 oxidation is carried out by methanotrophic bacteria in the surface soil and oxic soil-water interface zone [8]. In flooded soil CH_4 production is 2–4 times higher than the actual amount of CH_4 released under field conditions. This indicates that most of the CH_4 produced from soil is oxidized [9].

In the soil-water transition zone iron reduction-oxidation (IRO) and CH_4 oxidation occurs simultaneously. Several studies link IRO and CH_4 cycling process in anoxic wet land sediments [9,10]. In rice soil ecosystem Fe and Mn redox coupled CH_4 oxidation has been linked [11]. However, the interaction between Fe/Mn redox process and CH_4 oxidation is not adequately covered. In a previous study we have established that CH_4 oxidation is influenced by sequential reduction of terminal electron acceptors. Soil undergoing NO_3^- reduction, and Fe reduction stimulates CH_4 oxidation, while SO_4^{2-} reduction and methanogenesis inhibit CH_4 oxidation [10]. However, it is not known how the repeated iron reduction-oxidation

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* Corresponding author.
E-mail address: mohantywisc@gmail.com (S.R. Mohanty).

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cycle influences CH_4 oxidation in soil. Indian agriculture is mostly rain-fed and during wet monsoon soil undergoes flooding and drying continuously. In rice cultivation it is recommended to practice alternate flooding and drying to improve crop yield and water use efficiency [12]. These activities create reduction-oxidation cycling in soil. In this study two soil types (vertisol and alluvial) were used to investigate and understand how iron-reduction-oxidation cycling influences CH_4 oxidation. Both soils differed in their physico-chemical and redox properties, one representing more oxic and the other more anoxic conditions.

2. Materials and methods

2.1. Soil sampling sites characterization

Soil samples were collected from two sites located at the experimental farm of the Indian Institute of Soil Science (IISS), Bhopal and Indian Agricultural Research Institute (IARI), N Delhi during June 2015. At Bhopal, soils were collected from the experimental fields (23.30 N, 77.40 E) of the national network project on organic farming. The location has a humid subtropical climate, with a hot summer and a humid monsoon season. It experiences southwestern monsoon rain between July and September. The soil (0–10 cm depth) used in this experiment was from the control field (unfertilized) cultivated with soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.) since 2004. The soil is clayey vertisol subgroup typic Haplustert. It is characterized with organic C 5.7 g kg^{-1}, available N 225 mg kg^{-1} and available P 2.6 mg kg^{-1} but high in available K (230 mg kg^{-1}). The textural composition of soil was: sand 15.2%, silt 30.3%, clay 54.5%. The soils electrical conductivity (EC) was 0.43 dS m^{-1}, and the pH value was 7.5 [13]. The IARI field site is located at 28.37 N and 77.11 E. The climate of Delhi is semi-arid and sub-tropical, characterized by hot summers and cold winters. The soil (0–10 cm depth) used in this experiment was collected from the unfertilized control field of rice (*Oryza sativa* L.) and rice cropping system since 2002. The soil is of alluvial origin, sandy clay loam in texture, alkaline in reaction and bears low cation exchange capacity. It belongs to the hyperthermic family of Typic Haplustept with organic carbon 5.0 g kg^{-1}, available N mg 140 kg^{-1}, available P 2.5 mg kg^{-1}, available K 140 mg kg^{-1}, pH 7.5–8.5, and EC 0.4–0.5 dS m^{-1} [14]. After collection, the soil samples were air dried under shade and after breaking the clods, soils were passed through a 2 mm mesh sieve. Soils were used for this experiment within 2 d after sampling.

2.2. Microcosm set-up and iron reduction-oxidation cycle

For the incubation experiment 10 g soil was placed to 130 mL autoclaved serum bottles and closed with neoprene septa. Slurries were prepared by adding 25 mL of distilled water. A total of thirty bottles were prepared for this experiment. Twenty four bottles (two soils x four Fe reduction-oxidation time points x three replicates) were used for iron reduction-oxidation cycling (IRO bottles). Six bottles (two soils x three replicates) were used for monitoring the Fe reduction and oxidation process (test bottles). The bottles were placed in completely randomized design. Before incubation six "IRO bottles" were incubated for CH_4 oxidation. These bottles represented the "control". All other bottles (18 IRO bottles and 6 test bottles) were kept at 30 ± 2 °C in an incubator with shaking at 100 rotations per minute (rpm).

Temporal variation of the iron reduction-oxidation in soil samples was monitored in the "test bottles". At regular intervals, 0.1 mL slurry sub samples were withdrawn and the concentration of Fe^{2+} and SO_4^{2-} was measured as described below. At 10 d of incubation Fe^{3+} reduction rate was high and there was no apparent SO_4^{2-} reduction. At this time point, all the bottles (both IRO and test bottles) were opened and shaken aerobically to completely oxidize Fe^{2+}. When the Fe^{2+} concentration was 0, all the bottles were again re-sealed with neoprene septa after adding equivalent volume of sterile distilled water that was evaporated during shaking. The process of Fe^{3+} reduction followed by Fe^{2+} oxidation is referred as iron reduction-oxidation (IRO) cycle. The iron reduction-oxidation cycle observed for first time was called IRO-1. Similarly, IRO was repeated for 2nd and 3rd times. The respective set of bottles was called IRO-2 or IRO-3. These IRO bottles were incubated to determine CH_4 oxidation rate. Temporal variation of IRO and the time points when the slurries were incubated for CH_4 oxidation are indicated in Fig. 1.

2.3. Reduced Fe, total extractable Fe and SO_4^{2-} analysis

Reduced Fe^{2+} in slurry samples was extracted with 0.5 N HCl and ferrozine assay [2]. Briefly, 0.5 mL slurry samples were withdrawn from serum bottles and placed into 10 mL vials containing 4.5 mL 0.5 N HCl. The vials were closed with a rubber septum and shaken for 1 h on a rotary shaker at 100 rpm. Then they were centrifuged at 5000 rpm for 5 min. Aliquots of 1 mL was added to the ferrozine reagent (1 g of ferrozine per liter of 50 mM HEPES buffer). The absorbance was taken at 562 nm wavelength using a UV-VIS spectrophotometer (Systronics 2201, Ahmedabad, Gujrat). Total acid extractable Fe (Fe^{2+} + Fe^{3+}) in the slurry samples was analyzed by adding 0.25 M NH_2OH to the 0.5 N HCl extracts. Following hydroxylamine reduction, total acid-extractable Fe was measured by ferrozine assay [15]. SO_4^{2-} was estimated using $Ca(H_2PO_4)_2$ as extractant and turbidometric analysis [16]. Potential iron reduction rates were estimated during Fe^{3+} reduction phase, while potential iron oxidation rate during Fe^{2+} oxidation phase. Rates were calculated from the slope of linear fit of ln transformed Fe^{2+} values over the incubation period.

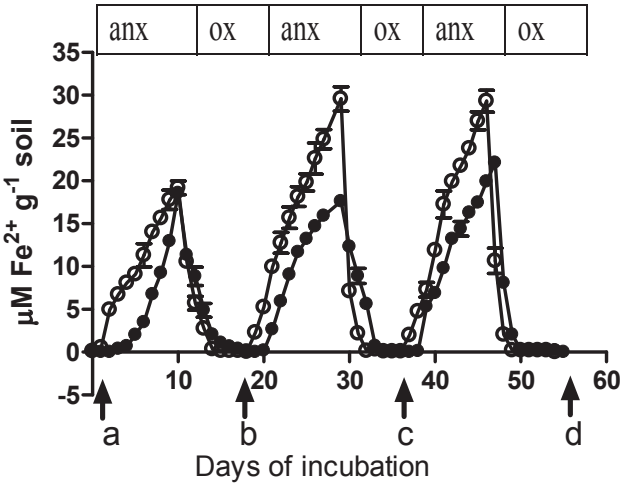

Fig. 1. Iron reduction-oxidation cycling process in a vertisol (closed circle) and an alluvial soil (open circle). The Y axis represents Fe^{2+} concentration in slurry samples. X axis represents incubation period in days. Temporal scale of anaerobic (anx) and aerobic (ox) incubation is shown on the top of figure. Arrows indicate the iron reduction-oxidation (IRO) cycling and sampling time points. IRO represents Fe^{3+} reduction followed by oxidation of Fe^{2+}. Time points are before incubation (a) and after different iron reduction-oxidation cycling IRO-1 (b), IRO-2 (c), and IRO-3 (d). CH_4 oxidation was measured at these time points. Each data point is arithmetic mean and standard deviation as error bar of three replicated observations.