BIOGEOCHEMICAL CYCLING OF GREENHOUSE GAS METHANE (CH_4) IN SOIL ECOSYSTEM

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Greenhouse gas CH_4 is the final product of anaerobic methanogenic respiration. Agriculture contributes about one third of total atmospheric CH_4 budget. The production of CH_4 by anaerobic methanogens includes reduction of methanol, CO_2 and cleavage of acetate, as well as biosynthesis of methylated compounds (Figure 1). Methanogenic archaea are two types: acetoclastic and hydrogenotrophic. These two groups of methanogens play a vital role for all biogenically produced CH_4 in anoxic habitats. Acetate is used as C source by the acetoclastic methanogenic archaea. These groups are represented as Methanosarcinaceae and Methanosaetaceae.

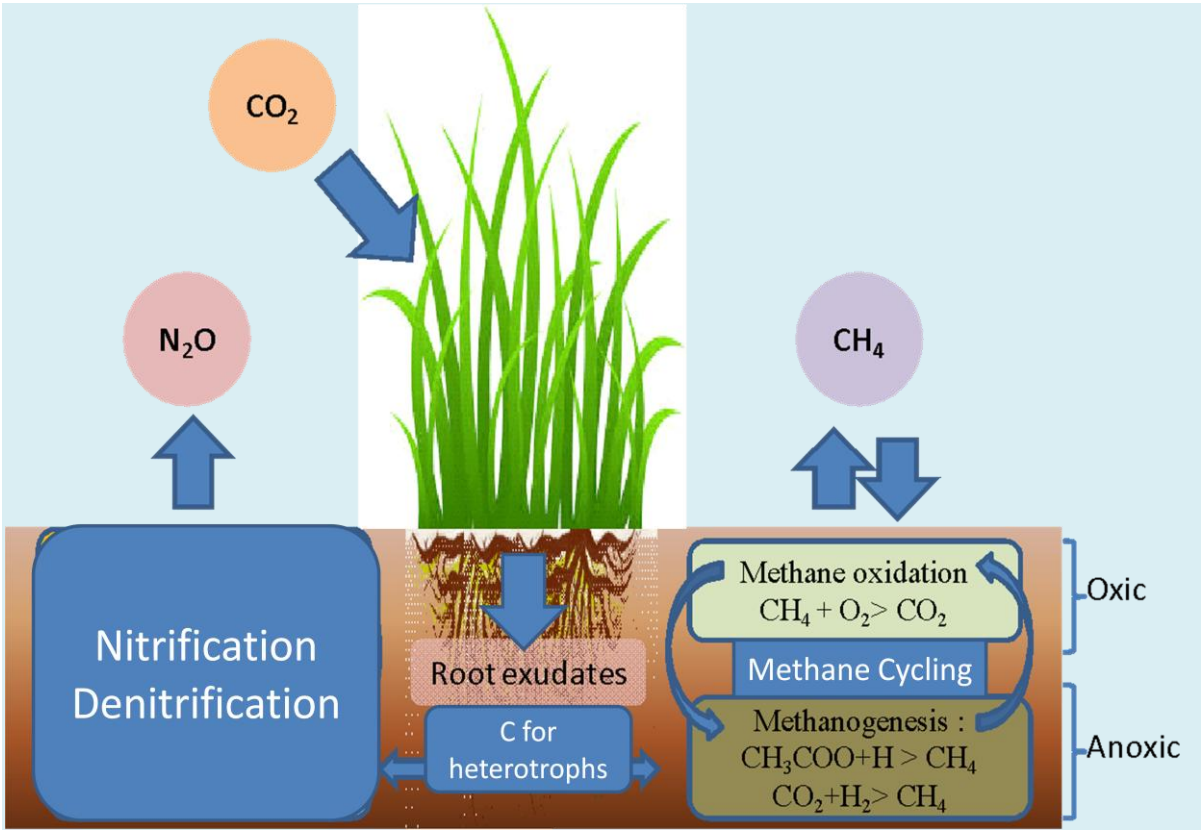

Figure1. Biogeochemical cycling of greenhouse gas methane (CH_4) in soil ecosystem

They are abundant in paddy soil when acetate concentration is low, while Methanosarcinaceae are dominant at higher acetate concentration. Hydrogenotrophic methanogens use H_2 and CO_2 for methanogenesis like Methanocellales, Methanomicrobiales, Methanosarcinales, and

Methanobacteriales. In wetlands CH_4 formation from H_2/CO_2 is much larger (up to 67%) than from acetate (33%). Net amount of CH_4 reaching the atmosphere is influenced by many abiotic factors including soil temperature, pH, nutrient content and moisture. Plants regulate the flux of CH_4 from wetlands by different processes. Plants can stimulate CH_4 emissions by providing carbon substrates to the methanogens. These C compounds originate from plants as root exudates. Plants also help in the transport of CH_4 from soil to atmosphere by acting as a conduit. Plants create oxidized condition in the rhizosphere that can influence CH_4 oxidation. The relative significance of these processes varies among plant species. Many studies have revealed that CH_4 fluxes can be increased by the presence of vascular plants, while others have found that there can be a decline in CH_4 production.

In general rice straw is incorporated into the soil during field preparation for cultivating rice. Amendment of rice straw to flooded paddy soil improves soil structure and soil organic carbon in the long term. However, such practice potentially increases CH_4 emission from soil into the atmosphere The decomposed organic matter acts as an electron source to reduce O_2, NO_3, Fe^{3+}, Mn^{4+}, SO_4^{2-} and CO_2 sequentially in the anaerobic soils. Presence of electron acceptors other than CO_2 i.e O_2, NO_3, Fe^{3+}, Mn^{4+}, SO_4^{2-} inhibits methanogenesis. Methanogens respond differently to the incorporated organic residues. It has been reported that rice straw incorporation into soil selectively enhances population of Methanosarcinaceae and Methanobacteriales, and decreases methanogens belonging to rice cluster I (RC-I) and Methanomicrobiales. The pattern of CH_4 production is, however, mainly dependent on the soil but not on the types of straw added.

METHANE OXIDATION

Upland soils are generally well-drained and aerated in nature. These soils have major role in the global CH_4 budget as they act as sink for atmospheric CH_4. It is estimated that globally CH_4 consumption is about 30 Tg yr^{-1}. CH_4 consumption in the pristine forest soils have been identified as the most promising sinks for atmospheric CH_4. Conversion of pristine forest land to agricultural land can lower the CH_4 uptake capacity. Various agricultural factors regulate CH_4 oxidation like soil compaction, pH and fertilizer application, abandonment of agricultural land or even converting it to forest can potentially increase the atmospheric CH_4 uptake to some extent.

Methanotroph diversity and activity has been studied in different upland soils. The diversity of CH_4 oxidizing bacteria are typically assessed by exploring *pmoA* gene which encodes β-subunit of methane monooxygenase (pMMO) enzyme. Most of the uncultivated methanotrophs are characterized by *pmoA* gene sequences. Similarly, methanotrophs can be identified by analyzing their phospholipid fatty acids. They are aerobic, gram-negative bacteria and use CH_4 as their sole source of energy. They also degrade various environmental contaminants and are used in various environmental remediation projects.Based on the physiological and biochemical characteristics of these microbial groups, the cultured members of the methanotrophs are divided into three groups: type I, type II, and type X. Type I are the members of the class γ-proteobacteria (*Methylomonas*, *Methylococcus*, *Methylomicrobium*, *Methylothermus*, *Methylohalobium*, *Methylocaldum* and *Methylobacter*). Type II belongs to the class α-proteobacteria (*Methylosinus*, *Methylocella*, *Methylocapsa* and *Methylocystis*). Type X methanotrophs belong to the class γ-proteobacteria (*Methylococcus)* and features characteristic of the both type I and II.

The first step of CH_4 oxidation is catalyzed by the enzyme methane monooxygenase (MMO) (Fig 2). This enzyme occurs as a membrane-bound particulate methane monooxygenase (pMMO) and cytoplasmic soluble methane monooxygenase (sMMO). Most of the methanotrophs (except *Methylocella*), possess pMMO. Certain Type II methanotrophs (*Methylosinus, Methylocystis*), Type I methanotrophs (*Methylomonas, Methylomicrobium*) and type X (*Methylococcus capsulata*) possess sMMO in addition to pMMO. The nucleotide sequence of the sMMO gene is constituted of *mmoX*, *mmoY*, *mmoB*, *mmoZ*, *mmoC* and *mmoD* . The DNA sequences of this cluster are highly conserved. These genes are used as genetic markers to identify enzymes of various methanotrophs. Methanol dehydrogenase (MDH) is the second enzyme involved in methane oxidation. It is present in all

methanotrophs including methane and methanol users. This enzyme is encoded by *mxaF* gene and is an appropriate marker for identifying methanotrophs possessing MDH activity.

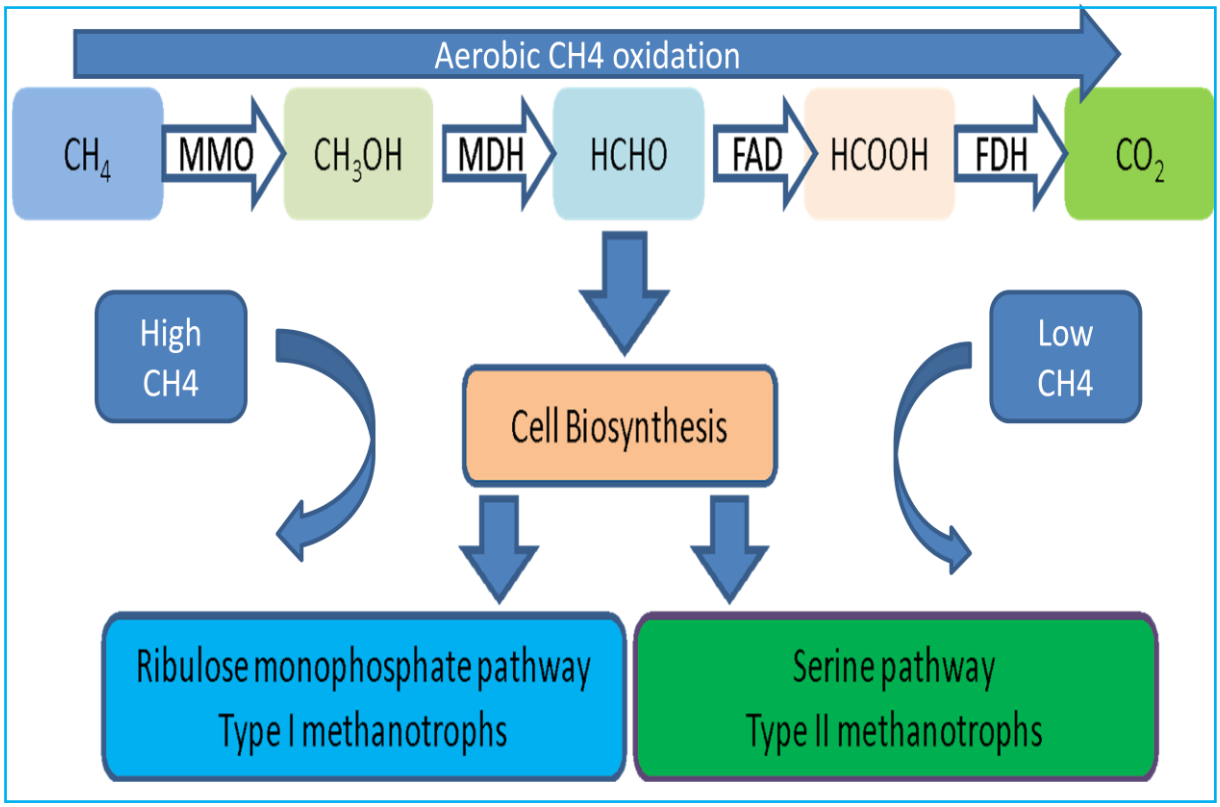

Figure 2. Schematic illustration of CH_4 oxidation pathway carried out by methanotrophs in soil. The enzyme complexes are methane monooxygenase (MMO), methanol dehydrogenase (MDH), formaldehyde dehydrogenase (FAD), and formic acid dehydrogenase (FDH).

AGRICULTURAL MANAGEMENT TO IMPROVE ATMOSPHERIC METHANE OXIDATION

Soil is the major biological sink for the atmospheric CH_4. The uncultivated methanotrophs are mostly responsible for atmospheric CH_4 (~1.8 ppm) consumption which is referred as 'high-affinity' methane oxidation (HAMO). The HAMO is carried out by the conventional methanotrophs commonly found in paddy soil. Low affinity CH_4 oxidation was carried out at high CH_4 concentration (10,000 ppm). The high affinity methanotrophic activity was lost over 2 weeks. However, the HAMO activity was regained by flush-feeding the soil with 10,000 ppm of CH_4. The induction of HAMO activity occurred only after the rapid growth of methanotrophs. Metatranscriptome analysis revealed a strong transcriptional activity of the key enzymes of the conventional methanotrophs. Conventional methanotrophs are responsible for atmospheric CH_4 oxidation if the soil undergoes alternate draining.

LINKAGE BETWEEN IRON REDOX CYCLING AND CH_4 CONSUMPTION

Our understanding on the relation between iron (Fe) reduction-oxidation (IRO) and CH_4 oxidation (consumption) is important to mitigate atmospheric CH_4. In an experiment, relation between the two biogeochemical processes examined in two soil types (alluvial and vertisol). Potential iron oxidation in both soils increased with repeated flooding and drying. The iron reduction-oxidation significantly stimulated CH_4 oxidation rate. The high affinity CH_4 oxidation rate (µg CH_4 consumed per g soil per day) increased from 0.03 to 0.19. Low affinity CH_4 oxidation rate increased from 0.05 to 0.47 in vertisol. X ray diffraction revealed that diffraction intensity of Fe minerals (magnetite and goethite) decreased over iron reduction oxidation cycle. Real time PCR quantification of methanotrophs (*pmoA* gene) confirmed that iron reduction oxidation cycle stimulated methanotroph abundance. The study

highlights that iron reduction-oxidation cycles can significantly enhance CH_4 oxidation in tropical soils . A high surface area of Fe minerals may change the soil environment to more aerobic and nutrient rich supporting the microbial activity. It is hypothesized that low crystalline Fe minerals act as micro-environments for bacterial activity. Probably, these altered properties of Fe minerals results after iron reduction-oxidation cycling which favoured methanotrophs and stimulated CH_4 oxidation.

ROLE OF AMMONIA OXIDIZING BACTERIA IN CH_4 CONSUMPTION

The lithoautotrophic ammonia oxidizing bacteria are important in terms of their role in greenhouse gas emission. These bacteria use ammonia as sole energy source and are able to fix CO_2 through the Calvin Benson cycle. The ammonia oxidizing bacteria are relevant for CH_4 consumption. Two main genera of ammonia oxidizers are *Nitrosomonas* and *Nitrosospira.* Both the genera belong to the β-proteobacteria. Nitrosococcus cluster belong phylogenetically within the γ-proteobacteria . The first step of nitrification is the oxidation of ammonia to hydroxylamine. This process is catalyzed by the ammonium monooxygenase. This enzyme is evolutionarily related to the methane monooxygenase enzyme (MMO). Often, the ammonium monooxygenase doesn't show high substrate specificity and oxidizes several compounds such as carbon monoxide and hydrocarbons. This enzyme is also capable of oxidizing CH_4, however, it occurs at much lower pace than the methane monooxygenase.

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