## Methanotrophs: the potential biological sink to mitigate the global methane load

Methane (CH<sub>4</sub>), a potent greenhouse gas (GHG), is involved in a number of chemical and physical processes in the earth's atmosphere, including global warming<sup>1</sup>. In the global CH<sub>4</sub> cycle, substantial amount of CH<sub>4</sub> is consumed by biological processes. The only known biological sink for atmospheric CH<sub>4</sub> is its oxidation in aerobic soils by methanotrophs or methane-oxidizing bacteria (MOB), which can contribute up to 15% to the total global CH<sub>4</sub> destruction<sup>2</sup>.

Methanotrophs, Gram-negative bacteria that utilize CH4 as their sole source of carbon and energy, play a crucial role in reducing global CH4 load due its CH4 consumption characteristics. Studies on CH<sub>4</sub> sink measurement from various agro- and natural ecosystems showed that the soils of these ecosystems exhibited a significant variations in CH<sub>4</sub> sink activity due to methanotrophic bacteria (Figure 1). Forest soil methanotrophic communities exhibit the highest CH4 sink activity on a global scale<sup>3</sup>. The conversion of forest ecosystem to agroecosystem decreases the methanotrophic CH<sub>4</sub> sink capacity, but it has been restored by afforestation. Recently, Dorr et al.<sup>4</sup> have reported that the clearing of a natural subtropical forest to farmland led to a reduction of CH<sub>4</sub> consumption and a shifting from one methanotrophic species to other species. Their data also suggest a restoration of the original community and the atmospheric CH<sub>4</sub> sink after reforestation. The CH<sub>4</sub> sink restoration is recorded faster in tropical regions than in temperate regions. Research on CH<sub>4</sub> flux measurement from dry tropical soils in India showed that these soils act as a strong sink of methane. Further, these soils exhibited a strong seasonality in the rates of CH<sub>4</sub> uptake. It may be argued that the variation in methane oxidation rates due to space and time could be due to fluctuation in the abundance and community composition of methanotrophs. It may be assumed that such soils may harbour different methanotroph population sizes in different seasons. If this is true for tropical soils, the seasonal variations in CH<sub>4</sub> sink activity by these soils may be described. Population size of methanotrophs in different ecosystems such as dry tropical forest and rainfed paddy soils could be an important factor that may affect the rate of methane oxidation. However, experimental evidences for these explanations are lacking for the above ecosystems and therefore, detailed studies are required to analyse the correlation between the CH<sub>4</sub> sink strength and methanotrophic population<sup>5</sup>.

A 60% increase in rice cultivation can be the most appropriate way to sustain the

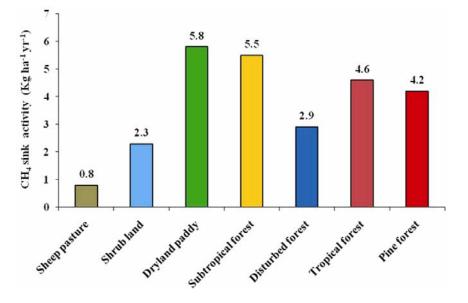


Figure 1. Methane sink activity performed by methanotrophs in the soil of different types of ecosystems. (Data has been modified from J. S. Singh<sup>2</sup>.)

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estimated increase of the human population during the next three decades and consequently, intensified global fertilizer application will be needed. Some reports demonstrated that the indiscriminate application of ammonium-based fertilizers can adversely affect the growth and multiplication of MOB and will result in the reduction of methane sink phenomenon. As a consequence, the expected intensification of rice production will most likely lead to increased atmospheric CH<sub>4</sub> emissions in the near future, if the proper mitigation strategies are not applied. However, many studies have shown that approximately 10-30% of CH<sub>4</sub> produced by methanogens in rice paddies is consumed by MOB associated with the roots of rice<sup>6</sup>. Thus, before the CH<sub>4</sub> is generated in deeper layers of flooded paddy fields which is released into the atmosphere, a significant concentration is subject to consumption by methanotrophs in the surface soil layer and the rhizosphere'.

Since the biological CH<sub>4</sub> sink phenomenon in the soil of different ecosystems is mostly carried out by obligate aerobic metahnotrophic bacteria, it is sensitive to environmental perturbations. Therefore, the factors that limit or even inhibit the activities of MOB may have major effects on the global CH<sub>4</sub> budget. Besides major limiting factors, i.e. CH<sub>4</sub> and oxygen, nitrogen (N) one of the limiting factors for rice yields, can also play an important role in CH4 oxidation and may become an inhibiting or stimulating factor for growth of methanotrophs. There is an ongoing discussion on the possible effects of NH<sub>4</sub><sup>+</sup>-N-based N-fertilizer on the consumption of methane by methanotrophs, depending on which environment it has been applied. Currently, there are many contradictory results reporting inhibition effects, stimulation effects or no effects of NH<sub>4</sub><sup>+</sup>-N-based N-fertilization on MOB. So far many mechanisms have been proposed for stimulation and inhibition effects on methane oxidation and methanotrophs, but none of them has yet been experimentally verified. Therefore, it is suggested that a re-evaluation of fertilizer practice and controversies regarding the influence of N-fertilization on CH<sub>4</sub> generation and consumption activities from paddy fields is necessary.

It is evident that at the level of the microbial community, N stimulates the growth and activity of methanotrophs and at the biochemical level, NH<sub>4</sub><sup>+</sup>-N inhibits CH<sub>4</sub> consumption because of competition for methane monoxygenase (CH<sub>4</sub> - oxidizing enzyme). Because of the similarity in size and structure of CH<sub>4</sub> and NH<sub>3</sub> molecules, and the relatively low specificity of the monoxygenase enzymes responsible, both methanotrophs and ammonia oxidizers can oxidize CH<sub>4</sub> and NH<sub>4</sub><sup>+</sup>. Furthermore, the intermediates and end-products of ammonia oxidation, i.e. hydroxylamine and nitrite can be toxic to MOB and may also lead to inhibition of the CH<sub>4</sub> sink. However, in rice fields methanotrophs have to compete with other microorganisms and also with the rice plant itself for Therefore, methanotrophs N. mav undergo severe nitrogen limitation as the plants grow, and application of N-fertilization may relieve nitrogen limitation and stimulate methanotrophic growth and activity. Although there are increasing number of studies related to the effects of N-fertilization on the CH4 sink phenomenon, the effects on the process-governing agents, i.e. the methanotrophic community, have not been studied in detail. The question arises whether the methanotrophic community structure plays any role in the response of the methanotrophs to different N availability. Therefore, extensive studies on the effect of different NH<sub>4</sub><sup>+</sup>-N-based N fertilizers on CH4 consumption and methanotrophic community structure at different growth stages of paddy crop in field conditions are required.

The population size of methanotrophic bacteria may be one of the important factors that govern the extent of  $CH_4$ consumption in saline or alkaline environments, particularly the saline, rainfed paddy fields<sup>8</sup>. The rainfed, saline paddy fields may assume importance in quantification of the methanotrophs, if substantial amount of  $CH_4$  is consumed by MOB in such soils. Saline paddy fields are important as salinity is known to affect most of the microbial activities. We have recently conducted an experiment to assess the impact of pyrite and farmyard manure (FYM) amendments on the population dynamics of methanotrophs in saline, rainfed paddy fields<sup>9</sup>. Our study has demonstrated that FYM and pyrite application enhanced the population of methanotrophs in saline paddy soil. In such soils, the long-term organic regimes can affect both the abundance and community composition of methanotrophs. Thus, pyrite, plant residues and other organic amendments could be important strategies for enhancing the CH<sub>4</sub> sink activity, abundance and diversity of methanotrophs in the alkaline paddy soils. This experiment was conducted with the application of FYM and pyrite only, but the importance of other organic amendments, green manure and biofertilizers (cyanobacteria) in the restoration of salt-affected paddy soils as well as enhancement in methane sink activity of methanotrophs cannot be ruled out. Therefore, further study in the area is urgently required to find out the strategies which are beneficial to enhance the methane consuming process by methanotrophs in large saline wastelands.

Studies have shown that due to imbalance between methane sources and sinks due various manmade activities in the past 200 years, the global atmospheric concentration of CH<sub>4</sub> has risen exponentially. However, according to a recent report of the Intergovernmental Panel on Climate Change, the rise in atmospheric CH<sub>4</sub> concentration had declined significantly during the last 2-3 years. But it is still not clear whether the decline rate is because of decrease in emission or increase in CH4 sink activity, because methanotrophs play an important role in the biogeochemical carbon cycle and in the control of global climate change by consuming significant amounts of methane<sup>10</sup>. However, it is still a challenge for the cultivation of methanotrophs, although various isolates were obtained in the extreme environments recently. Therefore, future research will be undoubtedly conducted using advance molecular techniques to identify the diversity and community composition of methanotrophs in different ecosystems and their contribution in  $CH_4$  sink activity. Further, it has been recently reported that a 10% increase in  $CH_4$  consumption rate may stabilize the current concentration of this potent GHG in the environment. Hence, it is necessary to adopt various strategies that may have practical importance in stimulating the methane sink activities by methanotrophs to mitigate the problem of global warming due to  $CH_4$ .

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