Chapter 22
Synergistic Interaction of Methanotrophs and Methylotrophs in Regulating Methane Emission

Vijaya Rani, Rajeev Kaushik, Sujan Majumder, A. T. Rani, Asha Arambam Devi, Pratap Divekar, Priyanka Khati, K. K. Pandey, and Jagdish Singh

Abstract The atmospheric methane concentration is increasing rapidly at the rate of around 10 ppb/year. A concerted effort is required to reduce methane emission. Methanotrophs possess methane monooxygenase enzyme system and can consume a major portion of the methane produced in the environment. These microbes play a major role in the single-carbon-driven microbial food web. Microbial interaction is an important component of microbial ecology studies, and its role in community functioning and various biogeochemical cycles still remains unclear. A synergistic interaction occurs between the methanotrophs and non-methane-utilizing methylotrophs (NUM) in the natural ecosystem. The intermediates produced by the methanotrophs can be used as a carbon source by the NUM and support its existence. On the other hand, NUM consumes toxic intermediates like methanol and formaldehyde of the methanotrophs and prolongs their growth. The consumption of the intermediates (methanol, formaldehyde and formate) of the methane utilization pathway by methylotrophs as a result of cross-feeding enhances the methane utilization rate of that ecosystem. Co-inoculation of methanotrophs and NUM in the natural habitat particularly paddy ecosystem can aid in the reduction of net methane emission. This chapter highlights the role of microbial interactions, particularly between methanotrophs and methylotrophs, that can be harnessed to mitigate methane emission from the methane-rich environment.

Keywords Methylotrophs · Methanotrophs · Cross-feeding · Methane · Methanol

V. Rani (✉) · S. Majumder · A. T. Rani · P. Divekar · K. K. Pandey
Division of Crop Protection, ICAR-Indian Institute of Vegetable Research, Varanasi, India

R. Kaushik · A. A. Devi
Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

P. Khati
ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

J. Singh
ICAR-Indian Institute of Vegetable Research, Varanasi, India

P. Bhatt et al. (eds.), *Microbial Technology for Sustainable Environment*,
https://doi.org/10.1007/978-981-16-3840-4_22

22.1 Introduction

Methane, the principal component of natural gas, is a colourless, odourless greenhouse gas (GHG) and contributes around 14% to the total greenhouse gas emission. Methane-rich environments like paddy fields, wetlands, sewage, landfills and digestive system of ruminants and termites possess huge diversity of methanogen, methanotrophs and other methylotrophs (Kirschke et al. 2013; Lee et al. 2014). Methanotrophs are those bacteria that can consume methane to meet their carbon and energy requirements before it gets released into the atmosphere and plays a major role in reducing net methane emission, thereby maintaining global carbon balance. On the other hand, methylotrophs are a diverse group of bacteria, yeast, fungi and archaea that can utilize C1 compounds like methanol, monomethylamine, dimethylamine, trimethylamine, methanesulfonate and dimethylsulfonate including methane as the sole source of carbon and energy. Traditional methanotrophs of the group Alpha- and Gammaproteobacteria widespread in Types I, II and X of methanotrophs with the prefix 'methylo' are well studied and investigated. Members of Betaproteobacteria of the genera *Methylophilus* (Madhaiyan et al. 2009), *Methylovorus* (Govorukhina and Trotsenko 1991) and *Methylibium* (Nakatsu et al. 2006) are recently recognized as methane oxidizers. Besides Proteobacteria, few members of the phylum Verrucomicrobia belonging to genera *Methylacidimicrobium* and *Methylacidiphilum* can also utilize methane (Op den Camp et al. 2009; Sharp et al. 2013; van Teeseling et al. 2014). Methylotrophs, on the other hand, cover all the three domains of microorganisms, viz. Bacteria, Archaea and Eukarya. Methylotrophs are microorganisms with a diverse group that besides utilizing methane (methanotrophs) also include those that can utilize other carbon substrates with no C–C bonds like methanol and other methylated compounds like methylamine (Chistoserdova et al. 2009). Since all methylotrophs cannot utilize methane, it can be said that all methanotrophs are methylotrophs but all methylotrophs are not methanotrophs. The ability to oxidize methanol has been reported in both prokaryotes and eukaryotes. Eukaryotic yeast belonging to genera *Candida*, *Pichia*, *Hansenula* and *Torulopsis* can utilize methanol as the sole carbon source (Negruța et al. 2010). The prokaryotic members capable of oxidizing methanol are spread across Alphaproteobacteria (*Methylobacteria*, *Hyphomicrobium*), Betaproteobacteria (*Burkholderia*, *Methylibium*, *Methyloversatilis*) and Gammaproteobacteria (*Clonothrix fusca*, *Beggiatoa*, *Pseudomonas*), Verrucomicrobia, Cytophagales, Bacteroidetes (*Flavobacterium*), Firmicutes (*Bacillus methanolicus*, *Paenibacillus*) and Actinobacteria (*Microbacterium*, *Gordonia*, *Arthrobacter* and *Mycobacterium*) (Rani et al. 2021b; Kolb 2009; Madhaiyan et al. 2010; Waturangi et al. 2011; Jhala et al. 2014; McTaggart et al. 2015; Macey et al. 2020).

Non-methane-utilizing methylotrophs (NUM) are known to co-occur with methanotrophs in the natural ecosystem and affect methane utilization rate. Modern-day techniques like stable-isotope probing have indicated that a synergistic interaction occurs between the methanotrophic and non-methane-utilizing methylotrophic community (Shiau et al. 2020; van Grinsven et al. 2020). NUM is

known to survive on methane-derived carbon particularly methanol and enhance the methane oxidation rate (Krause et al. 2017). Moreover, emergent properties like interaction-induced production of metabolites may arise when microorganisms interact leading to altered community functions otherwise not possible in the individual cells (Watrous et al. 2012; Abrudan et al. 2015). The transfer of metabolites from methanotrophs is not only restricted to NUM but to a wide range of microbial taxa as evident from the DNA-SIP study (Beck et al. 2013). These findings suggest that the assimilation of methane by methanotrophs in the methane-rich environment provides carbon to a diverse group of microbes (NUM and other heterotrophs) and sometimes to other life forms as well (Sanseverino et al. 2012; Oshkin et al. 2015; Yu et al. 2017).

22.2 Pathway for Methane Utilization

The unique ability of the methanotrophs to metabolize methane comes from the presence of methane monooxygenase (MMO) enzyme system. It's the first enzyme in the metabolic pathway of methanotrophs. MMO enzyme can be either housed in an intracytoplasmic membrane known as particulate MMO (pMMO) or suspended freely in the cytoplasm known as soluble MMO (sMMO). pMMO, a copper-containing, membrane-associated enzyme, is found in all the methanotrophs except for the genera *Methylocella* and *Methyloferula* (Theisen et al. 2005) but is less studied as it is membrane-associated when compared to sMMO. Both sMMO and pMMO enzyme can act on a wide range of substrates ranging from single carbon substrate, methane to as long as eight carbon compounds. They can act on alkane, alkenes, cycloalkanes and even halogenated derivatives (McDonald et al. 2006). Alkanes can be oxidized by a group of enzymes like cytochrome P450, alkane hydroxylases, sMMO and pMMO (Beilen and Funhoff 2005). However, among these, only sMMO and pMMO can act on methane. Some methanotrophs can produce both pMMO and sMMO, and their expression is regulated by copper concentration in the environment. pMMO is expressed under high copper-to-biomass ratios, whereas sMMO is expressed when the copper-to-biomass ratio is low (Murrell et al. 2000).

Methanol produced by the action of MMO is further acted upon by methanol dehydrogenase to produce formaldehyde. Methanol dehydrogenase (Mdh) is pyrroloquinoline quinone (PQQ)-containing NAD^+-dependent oxidoreductase enzyme (Anthony and Williams 2003). Formaldehyde produced by methylotrophs can be assimilated either by RuMP pathway (Type I) or by serine pathway (Type II). RuMP (ribulose monophosphate pathway) was earlier thought to be restricted to methylotrophic bacteria. However, they are now reported in various prokaryotic microorganisms and their role in formaldehyde fixation and detoxification has been established (Nobuo et al. 2006). Anaerobic methane oxidation by archaea differs in their mechanism to utilize methane. They utilize methane via reverse and modified methanogenesis pathway. Various intermediates of the methane oxidation pathway,

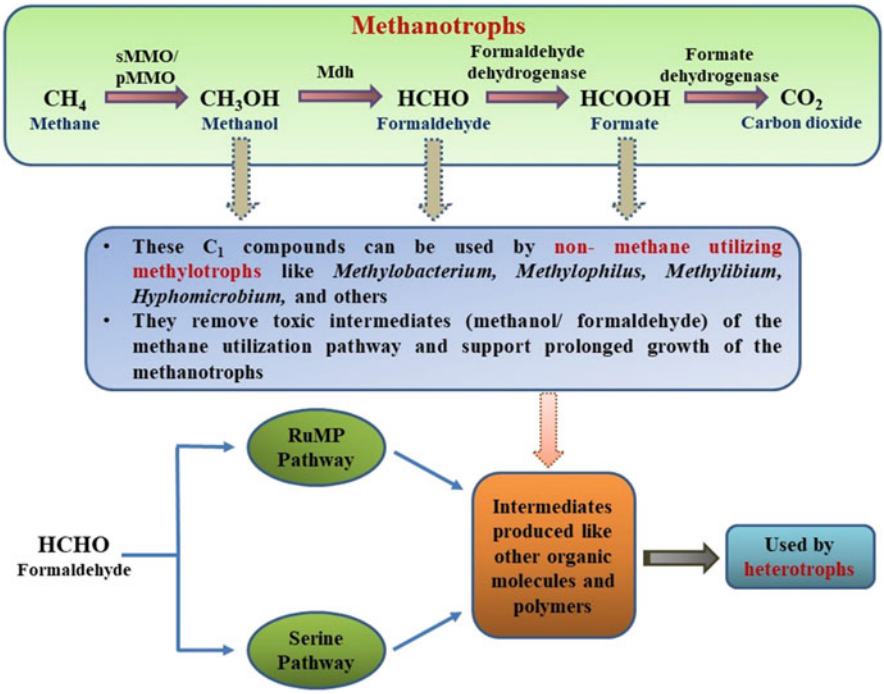

Fig. 22.1 Cross-feeding of metabolites produced by methanotrophs. *sMMO* soluble methane monooxygenases, *pMMO* particulate methane monooxygenases, *Mdh* methanol dehydrogenases, *RuMP* ribulose monophosphate pathway

viz. methanol, formaldehyde, formate, acetate and other organic acids when secreted by the methanotrophs, can be used as a growth substrate by both non-methane-utilizing methylotrophs and other heterotrophs as shown in Fig. 22.1. Methane-fed microbial microcosm study showed the abundance of methanotrophs of the family *Methylococcaceae* particularly *Methylobacter* along with other methylotrophs (*Methylotenera*) and heterotrophs, suggesting that there is a distribution of carbon from methane among diverse bacterial populations rather than a single type of microbe and thus methanotrophs play an important role in methane cycling (Oswald et al. 2016).

22.3 Cross-Feeding of Methane by NUM

Non-methanotrophs, particularly methylotrophs, play a major role in combating climate change in a methane-rich environment. Other heterotrophic forms may affect the growth of methanotrophic bacteria as a result of its various metabolic activities (secretion of growth factors or removal of toxic intermediates) (Hrsak and Begonja

2000). NUM is often known to coexist with methane-utilizing bacteria by cross-feeding on methane-derived carbon, particularly methanol (Takeuchi et al. 2019). Methane-oxidizing microorganisms possess monooxygenases that catalyze the conversion of methane to methanol. Methanol produced in the periplasmic space by the action of MMO enzyme system can easily diffuse out and serve as an alternative carbon source for other groups of microorganisms (Corder et al. 1986). Methanol-dependent cross-feeding between methanotrophs and other methylotrophs is largely dependent upon methanol excreted by the methanotrophic bacteria. Microflora residing in the rhizosphere, phyllosphere and non-rhizosphere or as endophytes of plants can utilize methanol and consume a major proportion of it (Kolb 2009; Iguchi et al. 2015; Chistoserdova and Kalyuzhnaya 2018). Methane-derived carbons (methanol, formaldehyde and formate) particularly methanol from methanotrophs can be utilized by NUM and enhance methane utilization rate by cross-feeding (Hanson and Hanson 1996; Qiu et al. 2008).

The findings of various researchers confirm the abundance of NUM along with methanogens and methanotrophs in the environment as shown in Table 22.1. Synergistic associations of methane and methanol oxidizers have been reported that favours the utilization of methane due to the removal of its intermediate methanol by the other partner (Krause et al. 2017; Jeong and Kim 2019). The coordinated response of *Methylococcaceae* (methanotroph) and *Methylophilaceae* (NUM) to changing methane and nitrate levels suggests that the two different functional groups of microbes may be involved in some type of cooperative behaviour (Beck et al. 2013). Similarly, methane oxidation by *Methylocystis* was found to increase in the presence of helper organism *Hyphomicrobium* according to the experiments carried out by (Jeong and Kim 2019). The transfer of methanol from the methanotrophic partner *Methylobacter tundripaludum* to the non-methanotroph methylotrophic partner *Methylotenera mobilis* has been confirmed in a microcosm model by (Krause et al. 2017). Their findings indicate that the non- methanotrophic partner induces a change in the gene expression of the methanotrophic partner causing the synthesis of less efficient methanol dehydrogenase enzyme (MxaF-type catalysing the conversion of methanol to formaldehyde) resulting in methanol excretion.

In the natural ecosystem, a complex interaction occurs between methanotroph, NUM and other heterotrophs. The success of single carbon-based microbial food web is determined by the effective transfer of intermediates from one microbial group to the other, allowing them to survive in methane-rich environments. A successful example of cross-feeding is the experiment carried out by Yu et al. (2017). They made a synthetic community of 50 bacterial cultures comprising of 10 methanotrophs (*Methylomonas*, *Methylobacter*, *Methylosarcina* and *Methylosinus*), 28 methanol-utilizing methylotrophs (*Methylotenera*, *Methylovorus*, *Methylophilus*, *Ancylobacter*, *Hyphomicrobium*, *Labrys*, *Methylobacteria*, *Methylopila*, *Paracoccus*, *Xanthobacter* and *Methyloversatilis*), 8 non-methanol-utilizing methylotrophs (*Aminobacter*, *Arthrobacter*, *Mycobacterium* and *Bacillus)* and 4 heterotrophs (*Pseudomonas*, *Janthinobacterium* and *Flavobacterium)* to study syntrophy in the aerobic methane-oxidizing environment (Yu et al. 2017). The metatranscriptomics analysis showed that across all the treatment with varying

Table 22.1 Studies showing cross-feeding of metabolites from methanotrophic to non-methane-utilizing methylotrophic partner

Methanotroph	Non-methanotroph	Substrate transferred	Salient findings	References
Methylobacter tundripaludum	*Methylotenera mobilis*	Methanol	Transcriptome analysis showed high expression of genes involved in methanol oxidation in the methylotrophic partner *Methylotenera mobilis* causes a change in the expression of methanotrophic partner causing it to secrete methanol	Krause et al. (2017)
Methylocaldum marinum	*Methyloceanibacter caenitepidi* (facultative methylotroph)	Acetate	Observed syntrophic association between *M. caenitepidi* and *M. marinum* Under co-culture condition, genes involved in serine pathway were downregulated in *M. caenitepidi* Organic compound probably acetate might be the major carbon source for the methylotrophic partner *M. caenitepidi*	Takeuchi et al. (2019)
Members of *Methylococcaceae* and others	*Methylophaga*	Not studied (may be methanol)	DNA-SIP experiment identified members of *Methylococcaceae* as major $^{13}CH_4$ consumers Microbial mats showed diverse assemblage of bacteria, protozoa with *Methylophaga* as key consumers of methane-derived organic matter	Paul et al. (2017)
Methylomicrobium	*Methylophaga*, *Hyphomicrobium* and other unrecognized methylotrophs	Not studied (may be methanol)	DNA-SIP study indicates that methane-derived carbon particularly methanol produced by methanotrophs may be consumed by *Methylophaga* and other related	Jensen et al. (2008)

(continued)

Table 22.1 (continued)

Methanotroph	Non-methanotroph	Substrate transferred	Salient findings	References
			uncultivated Gammaproteobacteria	
Methylococcaceae, particularly *Methylobacter*	*Methylophilaceae*, particularly *Methylotenera*	Not studied (may be methanol)	Observed coordinated response of both methanotroph and methylotroph to changing methane and nitrate levels, suggesting cooperative behaviour	Beck et al. (2013)
Methylobacter sp.	*Methylotenera*	Not studied (may be methanol)	No physical contact was required between the partners for the transfer of carbon as was confirmed by stable-isotope probing (SIP) and nanoscale secondary ion mass spectrometry (NanoSIMS) Requires nitrate for carbon transfer as it is potentially used by *Methylotenera* sp. and its deficiency may affect the methane oxidation rate of *Methylobacter* sp.	van Grinsven et al. (2020)
Methylococcaceae	*Methylophilaceae*	Methanol	Made synthetic bacterial communities of 50 isolates including methanotrophs, methylotrophs and heterotrophs with varying oxygen and methane levels Observed predominance of the methanotrophs of the family *Methylococcaceae* and non-methanotrophic methylotrophs of the family *Methylophilaceae* across all the treatments Vitamin B_{12} produced by *Methyloversatilis*	Yu et al. (2017)

(continued)

Table 22.1 (continued)

Methanotroph	Non-methanotroph	Substrate transferred	Salient findings	References
			may be shared among other community members	

nitrogen, oxygen and methane concentration, methanotroph of the family *Methylococcaceae* and methylotroph of the family *Methylophilaceae* did outcompete other species. Heterotrophs of the genera *Janthinobacterium* and *Pseudomonas* were detected in only a few treatments. Their research shows that methane-utilizing bacteria support the growth of other NUM and heterotrophs through the transfer of metabolites.

The bacterial community structure in a methane-rich environment is influenced by various factors like the existing concentration of methane, oxygen, nitrogen and other nutrients. The eutrophic lakes have high nitrate concentration and are one of the major sources of aquatic methane production. The nitrate in the aquatic ecosystem does influence the growth of microbial species and affect the cross-feeding of metabolites. The transfer of methane-derived carbon between *Methylobacter* (methanotroph) and *Methylotenera* (NUM) is based on the nitrate levels as it is required by the methylotrophic partner (van Grinsven et al. 2020). It has been observed that nitrate can cause stimulation in methane oxidation resulting in increased transfer of associated carbon compounds. Similarly, oxygen level selects the population of methanotrophs and methylotrophs, thereby determining their microbial diversity in a particular niche. The effect of oxygen on the conversion of methane-derived carbon has been studied (Wei et al. 2016). They observed greater transfer of methane-derived carbon at high O_2 concentration (21%) as compared to that observed at 2.5 and 5% O_2 concentration. They even reported higher microbial diversity index at 2.5% O_2 concentration and concluded that more methane-derived carbon was exuded into the environment and available for the growth of non-methanotrophs in O_2-limiting environments. Similar findings were reported where speciation within *Methylococcaceae* and *Methylophilaceae* family at different oxygen gradient with an abundance of *Methylosarcina* (methanotroph) and *Methylophilus* (NUM) at high O_2 tension (150–225 μM) and *Methylobacter* (methanotroph) and *Methylotenera* (NUM) at low initial O_2 tension (15–75 μM) was observed (Hernandez et al. 2015). The specific species differentiation observed within the methanotrophic and methylotrophic members of the *Methylococcaceae* and *Methylophilaceae* family is driven towards niche adaptation to specific oxygen gradient. The change in the population of methanotrophs and NUM to varying oxygen and methane concentration has been observed, suggesting that the relative concentration of methane and oxygen selects microbial community that can thrive under such situations. A synthetic community model comprising 50 bacterial species (methanotrophs, methylotrophs and heterotrophs) showed a change in the species composition with the abundance of methanotrophs of the family *Methylococcaceae*

and methylotrophs of the family *Methylophilaceae* at varying methane and oxygen concentration (Yu et al. 2017). Lanthanum (Ln), a rare earth metal, also affects the transfer of methane-derived carbon as it is an important co-factor of XoxF-type methanol dehydrogenases (MDHs) present in Gram-negative methylotrophs (Vu et al. 2016; Yanpirat et al. 2020). A shift in the expression of methanol dehydrogenases from lanthanide-dependent MDH (XoxF) type to the more efficient calcium-dependent MDH (MxaF) type occurs when non-methanotrophs are cultured along with methanotrophs, allowing an excess of methanol production that can be used by the methylotrophic partner (Krause et al. 2017). The presence of lanthanides allows a partner-induced change in gene expression and influences microbial interactions in the environment. The above finding suggests that the existing concentration of methane, oxygen, nitrate and other nutrients in the natural ecosystem plays a major role in determining the community composition of methanotrophs and methylotrophs, thereby influencing the transfer of methane-derived carbon and methane oxidation capacity of that particular ecosystem.

22.4 Approaches Used to Study the Interaction of Methanotrophs and NUM

Techniques involving the cultivation of different microbial groups cannot be very useful for interaction studies as it is difficult to simulate natural conditions under laboratory and most of the microorganisms still remain un-culturable due to their specific growth requirement. A useful approach is to simulate the natural environment under controlled condition through a microcosm or mesocosm experiment depending upon the scale of the model ecosystem and use molecular tools to determine community composition. Microcosms are artificial, controlled, simplified ecosystem used to simulate natural ecosystems mostly done under laboratory conditions, whereas mesocosms are bounded and partially enclosed outdoor experiment used to bridge the gap between the laboratory and the real world in environmental science (Bruckner et al. 1995). Microcosm and mesocosm experiment reduces the credibility gap and helps us to provide a solution to large-scale environmental problems. They provide a better understanding of the ecological problems by bringing them to spatial and temporal scale convenient enough to carry out the study (Benton et al. 2007). Microcosm experiments have widely been designed to study the diversity and dynamics of both methanotrophs and methylotrophs in soil and sediment samples collected from the natural environment (Shiau et al. 2020; Oshkin et al. 2015; Morris et al. 2002). Research shows that the activity pattern of methane-oxidizing bacteria and the population structure of methylotrophs follow the same pattern under field and microcosm condition (Eller et al. 2005). It can be concluded that the findings of the microcosm study can be extrapolated to field scale keeping in mind the concerned quantitative changes. Various molecular tools and

techniques are commonly being used to study the interaction of methanotrophs with NUM. Some of them are mentioned below.

DNA-Based Stable Isotope Probing (DNA-SIP): It is a powerful means to study the flow of intermediates from microbes with one functional group to the other. In DNA-SIP study, environmental samples are fed with substrate labelled with a heavy isotope (^{13}C). The labelled isotope then gets incorporated into the cell biomass including DNA, which can be processed and analysed to determine phylogenetic affiliations of species with labelled DNA. Isotope labelled $^{13}CH_4$ is used to study the cross-feeding of intermediates produced by methanotrophs determining the association of methanotrophs with methylotrophs and other heterotrophs in the natural environment. DNA-SIP helps us to establish a direct link between CH_4 oxidation and taxonomic identity for active methanotrophs and methylotrophs in complex environments (Shiau et al. 2020). It has been widely used to study metabolic interactions in methane-fed communities (van Grinsven et al. 2020; Paul et al. 2017; Jensen et al. 2008). DNA-SIP experiments are widely used to uncover the participants involved in the C1 cycle and give a clear picture of the transfer of metabolites from one microbe to the other. It provides confirmatory evidence of the associations of actively interacting microorganisms, sharing carbon derived from a single-key biogeochemical process.

PCR-Based Method: Functional marker genes unique to the physiology and metabolism of methanotrophs and methylotrophs can be targeted to study the diversity of microbes involved in the metabolism of single carbon compound. Functional genes commonly targeted to study the diversity of methanotrophs and methylotrophs are those of methane monooxygenases *(pmoA* and *mmoX)*, methanol dehydrogenase (*mxaF*), 16S rRNA region targeting serine pathway and RuMP, dinitrogen reductase (*nifH*) and formyltransferase/hydrolase complex (*fhcD*) (McDonald et al. 2008). PCR product can be run on denaturing gradient gel to separate amplicons even with a single-nucleotide difference. PCR followed by denaturing gradient gel electrophoresis (PCR-DGGE) will help us to determine the degree of genetic polymorphism in the target regions within the community (Bodelier et al. 2005; Piterina and Pembroke 2013). One major limitation of DGGE methodology is that the size of the amplicon should be between 100 and 500 bp, and therefore, primer set should be carefully designed (Marzorati et al. 2008). Eller et al. (2005) used three universal eubacterial primers set targeting methylotrophs with RuMP (533F/907R and 197F/533R) and serine pathway (142F/533R) followed by DGGE to study the community composition of methylotrophic bacteria in soil samples collected from the paddy field. The advantage of PCR-DGGE over DNA-SIP technique is that it does not require a closed controlled environment and can be used to determine community composition of samples directly collected from the natural environment.

Next-Generation Sequencing (NGS): Metagenomic and transcriptomic approach to study microbial diversity requires sequencing of a large amount of DNA and transcripts. Next-generation sequencing methods are more sensitive and can detect

low-frequency variants. It is a high-throughput process that handles hundreds and thousands of genes simultaneously and provides a comprehensive gene coverage (Krishna et al. 2019). Storage, analysis and interpretation of NGS data are the major rate-limiting steps of NGS technology. A large number of online bioinformatics tools are available that can process original raw sequencing data to functional biology (Kulski 2016). Techniques involving the use of NGS technology are widely used to study the interaction between methanotrophs and NUM (Krause et al. 2017; Beck et al. 2013; Takeuchi et al. 2019). Whole-genome sequencing and transcriptomic approach were used to study the interaction between the *Methylocaldum marinum* (methanotroph) and *Methyloceanibacter caenitepidi* (NUM) and observed that there is non-methanol-based cross-feeding (particularly acetate) of metabolites between the partners (Takeuchi et al. 2019). Pyrosequencing of 16S rRNA gene (27F/519R) was done to study the community dynamics in methane-fed microbial microcosms (Oshkin et al. 2015). The result showed low species diversity with the predominance of *Methylococcaceae* species, closely related to *Methylobacter tundripaludum* with few members of *Methylotenera*, *Flavobacterium*, *Pseudomonas*, *Janthinobacterium*, *Achromobacter* and *Methylophilus*. They also studied the community dynamics through Illumina sequencing of prepared DNA libraries and observed the predominance of methanotroph (*Methylobacter*) followed by NUM of the family *Methylophilaceae* (*Methylobacter tundripaludum*, *Methylophilus methylotrophus*, *Methylotenera versatilis* and *Methylotenera mobilis*). Both these techniques confirmed the strong correlation of the population of methanotrophs to that of NUM, suggesting that there may be the flow of intermediates between the two partners.

22.5 Interaction of Methanotrophs with Microbes of Different Functional Group

Besides methylotrophs, intermediates of the methanotrophic bacteria also support the growth of few heterotrophic bacteria. Synergistic interactions occur between the methanotrophs and heterotrophs where one provides the other with carbon source and the other produces growth factor or remove toxic intermediates from the environment (Stock et al. 2013; Ho et al. 2014; Veraart et al. 2018; Singh et al. 2019). Growth stimulation of methane-utilizing *Methylovulum miyakonense* in the presence of *Rhizobium* has been documented (Iguchi et al. 2011). They identified cobalamin secreted by *Rhizobium* as the key factor responsible for stimulating the growth of the methanotroph. Removal of toxic intermediates like organic acids can also support the growth and proliferation of methanotrophic partners (Singh et al. 2019). The effect of the interaction of methanotrophs with non-methanotrophs (heterotrophs/ autotrophs) has been summarized in Table 22.2.

Methanotrophic bacteria can grow with other organisms and aid in the removal of other greenhouse gas (Singh et al. 2019). Co-culture of alkaliphilic methanotrophic

Table 22.2 Beneficial effect of the interaction of methanotrophs with non-methanotrophs

Methanotroph	Non-methanotroph	Effect of interaction	References
Gammaproteobacteria (*Methylosarcina* and *Methylocaldum*)	Algae (autotroph)	Autotrophs provide O_2 to the methanotrophs and increase methane oxidation rate, whereas methanotrophs provide them CO_2 for photosynthesis	Yoshida et al. (2014)
Methylocystis	*Sphagnum* mosses (autotroph)	The autotrophs provide O_2 to the methanotrophs and increase methane oxidation rate, whereas methanotrophs provide them CO_2 for photosynthesis	Kip et al. (2011)
Methylobacter luteus	*Pseudomonas mandelii* (heterotroph)	Growth stimulation and increased methane oxidation	Veraart et al. (2018)
Methylovulum, *Methyloparacoccus*, *Methylomonas*	*Rhizobium* sp., *Mesorhizobium* sp. and *Sinorhizobium* sp. (heterotroph)	Heterotrophs produce vitamin B_{12} and support the growth of methanotrophs	Hoefman et al. (2014)
Methylomonas methanica	*Rhizobium/ Ochrobactrum/Pseudomonas/Escherichia coli* (heterotroph)	Growth promotion	Ho et al. (2014)
Methylovulum miyakonense	*Rhizobium* sp. (heterotroph)	Growth stimulation	Iguchi et al. (2011)
Methylomonas	*Cupriavidus taiwanensis* (heterotroph)	Heterotroph could synthesize quinone, pyridoxine and vitamin B_{12} and supported the growth of methanotroph	Stock et al. (2013)

bacteria with microalga *Scenedesmus obtusiusculus* in the ratio 3:1, 4:1 and 5:1 can lead to complete CH_4 and CO_2 uptake and thus is a promising strategy for greenhouse gas mitigation in a single step (Ruiz-Ruiz et al. 2020). Methanol-independent cross-feeding occurs in the natural ecosystem and supports the existence of non-methylotrophic heterotrophic bacteria. A recent study shows that methane-oxidizing bacteria can undergo mixed acid fermentation under the anoxic condition and release other products like acetate, succinate and H_2 (Kalyuzhnaya et al. 2013; Xin et al. 2004). These fermentation products can be used as a substrate by a diverse group of heterotrophic bacteria. The complex interaction of methanotrophs with other microbes occurs in the natural environment and thus can greatly influence net methane emission from these areas.

22.6 Importance of Interaction of Methane Utilizers with Non-methanotrophs in the Natural Ecosystem

Methanotrophs allow microbial food web to work at locations where it is difficult for other microbes to survive and consume methane which is the most reduced form of carbon. At the oxic-anoxic interface, aerobic methanotrophs survive that consume methane produced by methanogenic archaea and support the growth of other methylotrophs as well as heterotrophs. The type of interaction between these microbial functional groups in a methane-rich environment has been shown in Fig. 22.2.

Methylotrophic partner removes toxic intermediates of the methane utilizers like methanol and formaldehyde and allows sustained growth of the methanotrophs. Reports on excretion of methanol (up to 100 μM) in the culture medium are available that suggests a mismatch between the methanol produced and methanol that can be further assimilated into the cell biomass (Xin et al. 2004; Tavormina et al. 2017). The release of methanol will decrease the methane oxidation rate and inhibit methanol production by the methanotrophic culture. The presence of methanol-utilizing methylotrophs will allow removal of the released methanol and allow the sustained activity of methane monooxygenase enzyme system. Low methanol concentration in the environment is associated with low ozone concentration in the atmosphere and thus plays an important role in atmospheric chemistry (Warneke et al. 1999; Galbally and Kirstine 2002). Methanol-utilizing methylotrophs thereby play a key role and consume both plant-derived methanol and those obtained from methanotrophs

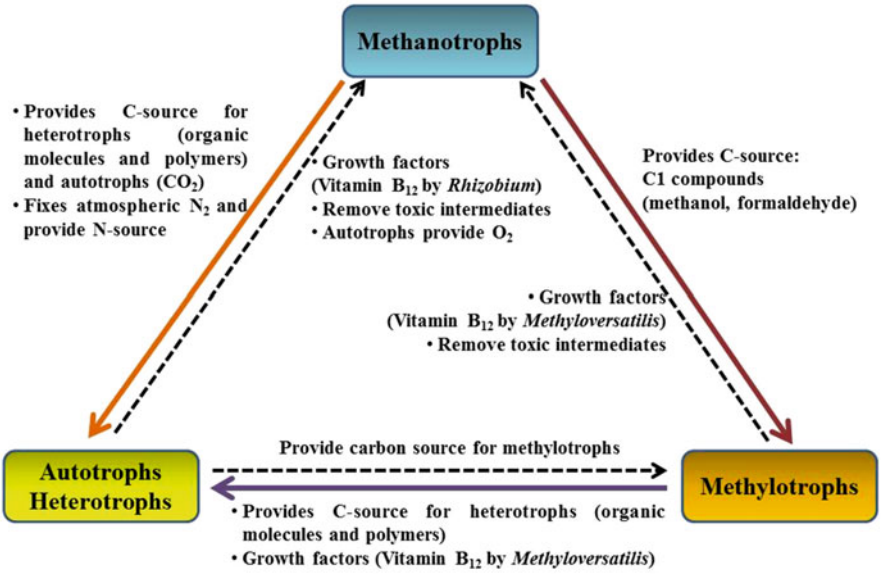

Fig. 22.2 The effect of the interaction of methanotrophs with non-methanotrophs (methylotrophs/heterotroph/autotroph) in a methane-rich environment

before it gets released into the atmosphere. Isolation of proteobacterial methanotroph requiring lanthanides by enrichment culture technique led to co-isolation of non-methanotrophic community, belonging to the genera *Methylophilus*, *Methyloversatilis*, *Hyphomicrobium*, *Methylobacteria*, *Pseudomonas* and *Thiobacillus*, as they can utilize intermediate compounds of the methane oxidation like methanol, acetate, and formate (Kato et al. 2020). Mesocosm experiments showed that there is a relative abundance of methanotrophs and NUM, indicating that a large part of methane-derived product (methanol, acetate and others) was being transferred from methanotrophs to non-methane-utilizing methylotrophs (Kuloyo et al. 2020).

In a natural environment, methanotrophs are found along with other methylotrophs, heterotrophs and autotrophs. Metabolites produced by each one of them may support or suppress the growth of other bacteria. Besides methylotrophs, heterotrophs and autotrophs also affect the activity of methane-utilizing bacteria. Growth factors (quinone, pyridoxine and vitamin B_{12}) produced by these organisms may support the activity of methanotrophs (Stock et al. 2013; Ho et al. 2014; Hoefman et al. 2014). Research shows that synergistic interaction exists between methanotrophs, methylotrophs and heterotrophs. A methane-utilizing mixed culture composed of a methanotroph, methanol-utilizing methylotroph (*Methyloceanibacter caenitepidi*) and a heterotroph was successfully established from the sample collected from marine sediments in Japan (Takeuchi et al. 2014). The stable association of these three functional groups on a medium with methane as carbon source shows that the methanotrophs via providing its metabolic intermediates (methanol, formaldehyde, acetate and formate) support the growth of methylotrophs as well as other heterotrophs in the environment. The close association of methane-oxidizing bacteria with autotrophs (macrophytic algae/*Sphagnum* mosses) suggests that their photosynthetic activity may provide O_2 to the methanotrophs and support its growth and proliferation (Yoshida et al. 2014; Kip et al. 2011). In turn, the methanotrophs may provide fixed nitrogen (NH_4^+) to the *Sphagnum* mosses by its N_2 fixation activity and exert beneficial effect (Larmola et al. 2014). Research suggests that the flow of methane-derived carbon does not stop at the microbial level but sometimes extend to the whole aquatic food web, up to the fish level (Sanseverino et al. 2012). Their findings indicate the importance of methanotrophs in the C1 cycle (particularly methane) and the role it plays in the food web of aquatic systems. Natural methane-rich environments possess a diverse group of microflora right from methanogens to methylotrophs, heterotrophs and autotrophs in close association, thereby allowing the microbial community to thrive.

22.7 Conclusion and Future Prospects

Studies have emphasized the importance of biotic interactions, particularly microbial interactions, as key modulators of biogeochemical processes. Methanotrophs along with other microbes allow methane-based food web to function in various anaerobic

ecosystems. Mitigation of methane emission through the use of methane-utilizing bacteria from various anthropogenic sources (paddy fields, wastewater treatment and landfills) has gained impetus in recent years (Oswald et al. 2016; Strong et al. 2017; Davamani et al. 2020). With the increase in anthropogenic methane emissions, the importance of these bacteria is set to increase as they play an important role in reducing global methane sink. Artificial inoculation of methanotrophs with plant growth-promoting traits in paddy field can cause a substantial reduction in methane emission and an increase in grain yield (Rani et al. 2021a; Davamani et al. 2020). Removal of methane from anoxic lake waters upon inoculation with γ-proteobacterial methanotrophs has been reported (Oswald et al. 2016). However, efforts to harness the synergistic interaction of methanotrophs with other microbial groups have not been undertaken. We propose that co-inoculation of NUM with methanotrophs may expedite the methane removal process due to their synergistic interaction. Studies in this area have still not gained impetus, and the effect of microbial co-inoculation on the removal of methane has still not been explored much. This chapter provides enough evidence and confirms the transfer of metabolites from methanotrophs to the other microbial groups. This microbial synergistic interaction can be tapped for reducing methane emission from various anoxic habitats.

References

Abrudan MI, Smakman F, Grimbergen AJ, Westhoff S, Miller EL, Van Wezel GP, Rozen DE (2015) Socially mediated induction and suppression of antibiosis during bacterial coexistence. Proc Natl Acad Sci 112(35):11054–11059

Anthony C, Williams P (2003) The structure and mechanism of methanol dehydrogenase. Biochim Biophys Acta 1647:18–23

Beck DAC, Kalyuzhnaya MG, Malfatti S, Tringe SG, Rio TG, Ivanova N, Lidstrom ME, Chistoserdova L (2013) A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae*. PeerJ 1:e23

Beilen JB, Funhoff EG (2005) Expanding the alkane oxygenase toolbox: new enzymes and applications. Curr Opin Biotechnol 16:308–314

Benton TG, Solan M, Travis JM, Sait SM (2007) Microcosm experiments can inform global ecological problems. Trends Ecol Evol 22(10):516–521

Bodelier PL, Meima-Franke M, Zwart G, Laanbroek HJ (2005) New DGGE strategies for the analyses of methanotrophic microbial communities using different combinations of existing 16S rRNA-based primers. FEMS Microbiol Ecol 52(2):163–174

Bruckner A, Wright J, Kampichler C, Bauer R, Kandeler E (1995) A method of preparing mesocosms for assessing complex biotic processes in soils. Biol Fertil Soils 19(2):257–262

Chistoserdova L, Kalyuzhnaya MG (2018) Current trends in methylotrophy. Trends Microbiol 26 (8):703–714

Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME (2009) The expanding world of methylotrophic metabolism. Annu Rev Microbiol 63:477–499

Corder RE, Johnson ER, Vega JL, Clausen EC, Gaddy JL (1986) Biological production of methanol from methane. Am Chem Soc Energy Fuels Div 33(3):469–478

Davamani V, Parameswari E, Arulmani S (2020) Mitigation of methane gas emissions in flooded paddy soil through the utilization of methanotrophs. Sci Total Environ 726:138570

Eller G, Krüger M, Frenzel P (2005) Comparing field and microcosm experiments: a case study on methano- and methylotrophic bacteria in paddy soil. FEMS Microbiol Ecol 51(2):279–291

Galbally IE, Kirstine W (2002) The production of methanol by flowering plants and the global cycle of methanol. J Atmos Chem 43(3):195–229

Govorukhina NI, Trotsenko YA (1991) *Methylovorus*, a new genus of restricted facultatively methylotrophic bacteria. Int J Syst Bacteriol 41(1):158–162

van Grinsven S, SinninghеDamsté JS, Harrison J, Polerecky L, Villanueva L (2020) Nitrate promotes the transfer of methane-derived carbon from the methanotroph *Methylobacter* sp. to the methylotrophs *Methylotenera* sp. in eutrophic lake water. Limnol Oceanogr. https://doi.org/10.1002/lno.11648

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Hernandez ME, Beck DA, Lidstrom ME, Chistoserdova L (2015) Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. PeerJ 3:e801

Ho A, De Roy K, Thas O, De Neve J, Hoefman S, Vandamme P, Heylen K, Boon N (2014) The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J 8:1945–1948

Hoefman S, van der Ha D, Iguchi H, Yurimoto H, Sakai Y, Boon N, Vandamme P, Heylen K, De Vos P (2014) *Methyloparacoccus murrellii* gen. nov., sp. nov., a methanotroph isolated from pond water. Int J Syst Evol Microbiol 64(6):2100–2107

Hrsak D, Begonja A (2000) Possible interactions within a methanotrophic-heterotrophic groundwater community able to transform linear alkylbenzenesulfonates. Appl Environ Microbiol 66 (10):4433–4439

Iguchi H, Yurimoto H, Sakai Y (2011) Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl Environ Microbiol 77:8509–8515

Iguchi H, Yurimoto H, Sakai Y (2015) Interactions of methylotrophs with plants and other heterotrophic bacteria. Microorganisms 3(2):137–151

Jensen S, Neufeld JD, Birkeland NK, Hovland M, Murrell JC (2008) Methane assimilation and trophic interactions with marine *Methylomicrobium* in deep-water coral reef sediment off the coast of Norway. FEMS Microbiol Ecol 66(2):320–330

Jeong S, Kim TG (2019) Development of a novel methanotrophic process with the helper microorganism *Hyphomicrobium* sp. NM3. J Appl Microbiol 126(2):533–544

Jhala YK, Vyas RV, Shelat HN, Patel HK, Patel KT (2014) Isolation and characterization of methane utilizing bacteria from wetland paddy ecosystem. World J Microbiol Biotechnol 30 (6):1845–1860

Kalyuzhnaya MG, Yang S, Rozova ON, Smalley NE, Clubb J, Lamb A, Gowda GN, Raftery D, Fu Y, Bringel F, Vuilleumier S (2013) Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat Commun 4(1):1–7

Kato S, Takashino M, Igarashi K, Kitagawa W (2020) Isolation and genomic characterization of a Proteobacterial methanotroph requiring lanthanides. Microbes Environ 35(1):ME19128

Kip N, Ouyang W, van Winden J, Raghoebarsing A, van Niftrik L, Pol A, Pan Y, Bodrossy L, van Donselaar EG, Reichart GJ, Jetten MS (2011) Detection, isolation, and characterization of acidophilic methanotrophs from Sphagnum mosses. Appl Environ Microbiol 77(16):5643–5654

Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, Bergamaschi P, Bergmann D, Blake DR, Bruhwiler L, Cameron-Smith P (2013) Three decades of global methane sources and sinks. Nat Geosci 6:813–823

Kolb S (2009) The quest for atmospheric methane oxidizers in forest soils. Environ Microbiol Rep 1 (5):336–346

Krause SM, Johnson T, Karunaratne YS, Fu Y, Beck DA, Chistoserdova L, Lidstrom ME (2017) Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. Proc Natl Acad Sci 114(2):358–363

Krishna BM, Khan MA, Khan ST (2019) Next-generation sequencing (NGS) platforms: an exciting era of genome sequence analysis. In: Microbial genomics in sustainable agroecosystems. Springer, Singapore, pp 89–109

Kuloyo O, Ruff SE, Cahill A, Connors L, Zorz JK, Hrabe de Angelis I, Nightingale M, Mayer B, Strous M (2020) Methane oxidation and methylotroph population dynamics in groundwater mesocosms. Environ Microbiol 22:1222–1237

Kulski JK (2016) Next-generation sequencing—an overview of the history, tools, and "omic" applications. In: Next generation sequencing-advances, applications and challenges. InTechOpen, London, pp 3–60

Larmola T, Leppänen SM, Tuittila ES, Aarva M, Merilä P, Fritze H, Tiirola M (2014) Methanotrophy induces nitrogen fixation during peatland development. Proc Natl Acad Sci 111(2):734–739

Lee HJ, Kim SY, Kim PJ, Madsen EL, Jeon CO (2014) Methane emission and dynamics of methanotrophic and methanogenic communities in a flooded rice field ecosystem. FEMS Microbiol Ecol 88(1):195–212

Macey MC, Pratscher J, Crombie AT, Murrell JC (2020) Impact of plants on the diversity and activity of methylotrophs in soil. Microbiome 8(1):1–17

Madhaiyan M, Poonguzhali S, Kwon ASAT (2009) *Methylophilus rhizosphaerae* sp. nov., a restricted facultative methylotroph isolated from rice rhizosphere soil. Int J Syst Evol Microbiol 59:2904–2908

Madhaiyan M, Poonguzhali S, Lee JS, Lee KC, Sundaram S (2010) *Flavobacterium glycines* sp. nov., a facultative methylotroph isolated from the rhizosphere of soybean. Int J Syst Evol Microbiol 60(9):2187–2192

Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10 (6):1571–1581

McDonald IR, Miguez CB, Rogge G, Bourque D, Wendlandt KD, Groleau D, Murrell JC (2006) Diversity of soluble methane monooxygenase-containing methanotrophs isolated from polluted environments. FEMS Microbiol Lett 255(2):225–232

McDonald IR, Bodrossy L, Chen Y, Murrell JC (2008) Molecular ecology techniques for the study of aerobic methanotrophs. Appl Environ Microbiol 74(5):1305–1315

McTaggart TL, Beck DA, Setboonsarng U, Shapiro N, Woyke T, Lidstrom ME, Kalyuzhnaya MG, Chistoserdova L (2015) Genomics of methylotrophy in Gram-positive methylamine-utilizing bacteria. Microorganisms 3(1):94–112

Morris SA, Radajewski S, Willison TW, Murrell JC (2002) Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Appl Environ Microbiol 68(3):1446–1453

Murrell JC, McDonald IR, Gilbert B (2000) Regulation of expression of methane monooxygenases by copper ions. Trends Microbiol 8:221–225

Nakatsu CH, Hristova K, Hanada S, Meng X, Hanson JR, Scow KM, Kamagata Y (2006) *Methylibium petroleiphilum* gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the Betaproteobacteria. Int J Syst Evol Microbiol 56:983–989

Negruța OANA, Csutak O, Stoica I, Rusu E, Vassu T (2010) Methylotrophic yeasts: diversity and methanol metabolism. Rom Biotechnol Lett 15:5369–5375

Nobuo K, Hiroya Y, Rudolf KT (2006) The physiological role of the ribulose monophosphate pathway in bacteria and archaea. Biosci Biotechnol Biochem 70(1):10–21

Op den Camp HJ, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MS, Birkeland NK, Pol A, Dunfield PF (2009) Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environ Microbiol Rep 1(5):293–306

Oshkin IY, Beck DA, Lamb AE, Tchesnokova V, Benuska G, McTaggart TL, Kalyuzhnaya MG, Dedysh SN, Lidstrom ME, Chistoserdova L (2015) Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. ISME J 9 (5):1119–1129. https://doi.org/10.1038/ismej.2014.203

Oswald K, Milucka J, Brand A, Hach P, Littmann S, Wehrli B, Kuypers MM, Schubert CJ (2016) Aerobic gammaproteobacterial methanotrophs mitigate methane emissions from oxic and anoxic lake waters. Limnol Oceanogr 61(S1):S101–S118

Paul BG, Ding H, Bagby SC, Kellermann MY, Redmond MC, Andersen GL, Valentine DL (2017) Methane-oxidizing bacteria shunt carbon to microbial mats at a marine hydrocarbon seep. Front Microbiol 8:186

Piterina AV, Pembroke JT (2013) Use of PCR-DGGE based molecular methods to analyse microbial community diversity and stability during the thermophilic stages of an ATAD wastewater sludge treatment process as an aid to performance monitoring. Int Scholarly Res Notices 2013:13

Qiu Q, Noll M, Abraham WR, Lu Y, Conrad R (2008) Applying stable isotope probing of phospholipid fatty acids and rRNA in a Chinese rice field to study activity and composition of the methanotrophic bacterial communities in situ. ISME J 2:602–614

Rani V, Bhatia A, Kaushik R (2021a) Inoculation of plant growth promoting-methane utilizing bacteria in different N-fertilizer regime influences methane emission and crop growth of flooded paddy. Sci Total Environ 775:145826

Rani V, Bhatia A, Nain L, Tomar GS, Kaushik R (2021b) Methane utilizing plant growth-promoting microbial diversity analysis of flooded paddy ecosystem of India. World J Microbiol Biotechnol 37(4):56

Ruiz-Ruiz P, Gomez-Borraz TL, Revah S, Morales M (2020) Methanotroph-microalgae co-culture for greenhouse gas mitigation: effect of initial biomass ratio and methane concentration. Chemosphere 259:127418

Sanseverino AM, Bastviken D, Sundh I, Pickova J, Enrich-Prast A (2012) Methane carbon supports aquatic food webs to the fish level. PLoS One 7(8):e42723

Sharp CE, den Camp HJO, Tamas I, Dunfield PF (2013) Unusual members of the PVC superphylum: the methanotrophic verrucomicrobia genus "*Methylacidiphilum*". In: Planctomycetes: cell structure, origins and biology. Humana Press, Totowa, pp 211–227

Shiau YJ, Lin CW, Cai Y, Jia Z, Lin YT, Chiu CY (2020) Niche differentiation of active methane-oxidizing bacteria in estuarine mangrove forest soils in Taiwan. Microorganisms 8(8):1248

Singh R, Ryu J, Kim SW (2019) Microbial consortia including methanotrophs: some benefits of living together. J Microbiol 57(11):939–952

Stock M, Hoefman S, Kerckhof FM, Boon N, De Vos P, De Baets B, Heylen K, Waegeman W (2013) Exploration and prediction of interactions between methanotrophs and heterotrophs. Res Microbiol 164(10):1045–1054

Strong PJ, Karthikeyan OP, Zhu J, Clarke W, Wu W (2017) Methanotrophs: methane mitigation, denitrification and bioremediation. In: Agro-environmental sustainability. Springer, Cham, pp 19–40

Takeuchi M, Katayama T, Yamagishi T, Hanada S, Tamaki H, Kamagata Y, Oshima K, Hattori M, Marumo K, Nedachi M, Maeda H (2014) *Methyloceanibacter caenitepidi* gen. nov., sp. nov., a facultatively methylotrophic bacterium isolated from marine sediments near a hydrothermal vent. Int J Syst Evol Microbiol 64(2):462–468

Takeuchi M, Ozaki H, Hiraoka S, Kamagata Y, Sakata S, Yoshioka H, Iwasaki W (2019) Possible cross-feeding pathway of facultative methylotrophs *Methyloceanibacter caenitepidi* Gela4 on methanotroph *Methylocaldum marinum* S8. PLoS One 14(3):e0213535

Tavormina PL, Kellermann MY, Antony CP, Tocheva EI, Dalleska NF, Jensen AJ, Valentine DL, Hinrichs KU, Jensen GJ, Dubilier N, Orphan VJ (2017) Starvation and recovery in the deep-sea methanotroph *Methyloprofundus sedimenti*. Mol Microbiol 103(2):242–252

van Teeseling MC, Pol A, Harhangi HR, van der Zwart S, Jetten MS, den Camp HJO, van Niftrik L (2014) Expanding the verrucomicrobial methanotrophic world: description of three novel species of *Methylacidimicrobium* gen. Nov. Appl Environ Microbiol 80(21):6782–6791

Theisen AR, Ali MH, Radajewski S, Dumont MG, Dunfield PF, McDonald IR, Dedysh SN, Miguez CB, Murrell JC (2005) Regulation of methane oxidation in the facultative methanotroph *Methylocella silvestris* BL2. Mol Microbiol 58:682–692

Veraart AJ, Garbeva P, Van Beersum F, Ho A, Hordijk CA, Meima-Franke M, Zweers AJ, Bodelier PLE (2018) Living apart together-bacterial volatiles influence methanotrophic growth and activity. ISME J 12:1163–1166

Vu HN, Subuyuj GA, Vijayakumar S, Good NM, Martinez-Gomez NC, Skovran E (2016) Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. J Bacteriol 198(8):1250–1259

Warneke C, Karl T, Judmaier H, Hansel A, Jordan A, Lindinger W, Crutzen PJ (1999) Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: significance for atmospheric HOx chemistry. Glob Biogeochem Cycles 13(1):9–17

Watrous J, Roach P, Alexandrov T, Heath BS, Yang JY, Kersten RD, van der Voort M, Pogliano K, Gross H, Raaijmakers JM, Moore BS (2012) Mass spectral molecular networking of living microbial colonies. Proc Natl Acad Sci 109(26):E1743–E1752

Waturangi DE, Francisca I, Susanto CO (2011) Genetic diversity of methylotrophic bacteria from human mouth based on amplified ribosomal DNA restriction analysis (ARDRA). Hayati J Biosci 18(2):77–81

Wei XM, He R, Chen M, Su Y, Ma RC (2016) Conversion of methane-derived carbon and microbial community in enrichment cultures in response to O_2 availability. Environ Sci Pollut Res 23(8):7517–7528

Xin JY, Cui JR, Niu JZ, Hua SF, Xia CG, Li SB, Zhu LM (2004) Production of methanol from methane by methanotrophic bacteria. Biocatal Biotransformation 22(3):225–229

Yanpirat P, Nakatsuji Y, Hiraga S, Fujitani Y, Izumi T, Masuda S, Mitsui R, Nakagawa T, Tani A (2020) Lanthanide-dependent methanol and formaldehyde oxidation in *Methylobacterium aquaticum* strain 22A. Microorganisms 8(6):822

Yoshida N, Iguchi H, Yurimoto H, Murakami A, Sakai Y (2014) Aquatic plant surface as a niche for methanotrophs. Front Microbiol 5:30

Yu Z, Beck DA, Chistoserdova L (2017) Natural selection in synthetic communities highlights the roles of *Methylococcaceae* and *Methylophilaceae* and suggests differential roles for alternative methanol dehydrogenases in methane consumption. Front Microbiol 8:2392