CHAPTER ONE

Microbial Ecology of Methanogens and Methanotrophs

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Contents

Rice agriculture feeds about a third of the world's population. However, rice fields are also an important source in the global budget of the greenhouse gas methane. The emission of methane from flooded rice fields is the result of the activity of methanogenic archaea that produce the methane and of methanotrophic bacteria that oxidize part of it, so that the ecology of these two physiological groups of microorganisms is key for the understanding of methane cycling in rice fields and for possible mitigation of emission from this important agro-ecosystem. In this chapter I will describe the ecology of methanogens and methanotrophs and will give examples where production and emission of methane on the field scale can be understood on the basis of processes on the microscale.

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1. Introduction

1.1. Global methane budget and processes controlling methane emission from rice fields

Methane is next to CO_2, the second most abundant carbon compound in the atmosphere. The mixing ratio of CH_4 in the atmosphere is presently about 1770 ppbv giving a global atmospheric burden of about 5000 Tg. The total budget of CH_4 is around 600 Tg a^{-1}, resulting in an atmospheric lifetime of about 8 years. Immediately after the ice age, the atmospheric mixing ratio of CH_4 was much lower, about 600 ppbv. After 1800 AD, however, CH_4 (like CO_2 or N_2O) started to increase dramatically and since then increased by about 0.5–1% per year. It is just since the last few years that the CH_4 mixing ratio seems to have stabilized at a relatively high level, which is about three times that after the ice age. Methane absorbs in the infrared spectrum of light, causing a greenhouse effect in addition to that by water vapor and CO_2 (Lacis *et al.*, 1981). Methane accounts for about 44% of the total anthropogenic radiative forcing due to changes in the concentrations of greenhouse gases and aerosols between 1850 and 2000, being about 0.7 W m^{-2} (Hansen *et al.*, 2000). On a molecular basis and a time frame of 100 years, the global warming potential of CH_4 is about 20 times stronger than that of CO_2. For pertinent literature and data see the home page of National Oceanic and Atmospheric Administration [NOAA (http://www.cmdl.noaa.gov/)] and the following references (Bousquet *et al.*, 2006; Chen and Prinn, 2005; Cicerone and Oremland, 1988; Lelieveld *et al.*, 1998; Reeburgh, 2003).

The global CH_4 budget is dominated by biogenic sources, natural wetlands (23%), and rice fields (21%) accounting for almost half of the total budget (Chen and Prinn, 2005). In these environments methane is exclusively produced by methanogenic microorganisms (Cicerone and Oremland, 1988; Conrad, 1989). Additional CH_4 sources for which methanogenic microorganisms are exclusively responsible are the intestines of ruminants and termites (20%), landfills, and other waste treatment systems (10%), so that about 75% of the total atmospheric CH_4 originates from the activity of methanogens (Chen and Prinn, 2005). Hence, methanogens, for example those in rice fields, contribute significantly to the global budget of the greenhouse gas methane.

The emission of CH_4 from biogenic sources would even be larger, if methanotrophic microorganisms would not attenuate the flux into the atmosphere by oxidizing part of the produced CH_4 (Reeburgh, 2003). Roughly estimated, about 1% of the primary productivity eventually results in CH_4 production, of which about half is emitted into the atmosphere, while the remainder is oxidized by methanotrophs (Reeburgh, 2003). From marine sediments, in particular, CH_4 emission would be substantially larger if

anaerobic methane-oxidizing microorganisms would not consume more than 75% of the CH_4, which is either produced from organic matter or is degassing from methane hydrate deposits (Reeburgh, 2003). It is probably because of the efficient attenuation by anaerobic methanotrophs that marine sediments are only a minor source in the atmospheric CH_4 budget. In freshwater wetlands and rice fields too, a substantial part of methane production is consumed by methanotrophs (Reeburgh, 2003). There, however, aerobic rather than anaerobic methanotrophs, which live at the interface between anoxic and oxic zones, are the important CH_4 consumers.

Aerobic methanotrophs are not only active in consuming the freshly produced CH_4, but can also utilize the CH_4 present in the atmosphere. The CH_4 is taken up from the atmosphere by aerated upland soils (Dunfield, 2007). In fact, methanotrophs in upland soils account for about 5% of the total sink of atmospheric CH_4, the remaining 95% being due to photochemical destruction of CH_4 and flux into the stratosphere (Reeburgh, 2003).

1.2. Role of methanogens and methanotrophs in carbon cycling and methane emission

In all the environments that act as biogenic sources for atmospheric CH_4, methane is produced by the same principle process, that is CH_4 is end product of the degradation of organic matter under anaerobic conditions. The methanogenic degradation of organic matter is accomplished by a complex microbial community (Conrad, 1989; Conrad and Frenzel, 2002). When for example degrading polysaccharides, members of the microbial community start hydrolyzing polysaccharides to sugars, which are subsequently fermented in a primary fermentation to various alcohols and fatty acids and to acetate, CO_2, and H_2 (Fig. 1). Only acetate or H_2 plus CO_2 are suitable substrates for methanogenic microbes, which convert these substrates to CH_4 plus CO_2 and CH_4 plus H_2O, respectively (Ferry, 1993). The other products of the primary fermentation, that is the alcohols and fatty acids, cannot be consumed directly by methanogenic microbes, but have to be converted to acetate, CO_2, and H_2 in a secondary fermentation, which is carried out by so-called syntrophic microorganisms. They are called syntrophs, since they can accomplish the degradation only in syntrophy with methanogens that immediately consume the formed H_2, which must not accumulate to partial pressures higher than a few pascal. Otherwise, the secondary fermentation would become thermodynamically endergonic and cannot proceed. Finally, the methanogenic community often consists of a further physiological group of fermenting bacteria, the so-called homoacetogenic bacteria (Drake, 1994). These bacteria ferment sugars directly to acetate as sole product. Some of the homoactogens, the so-called chemolithoautotrophic acetogens, are able to convert H_2 plus CO_2 to acetate. The entire pathway of organic matter

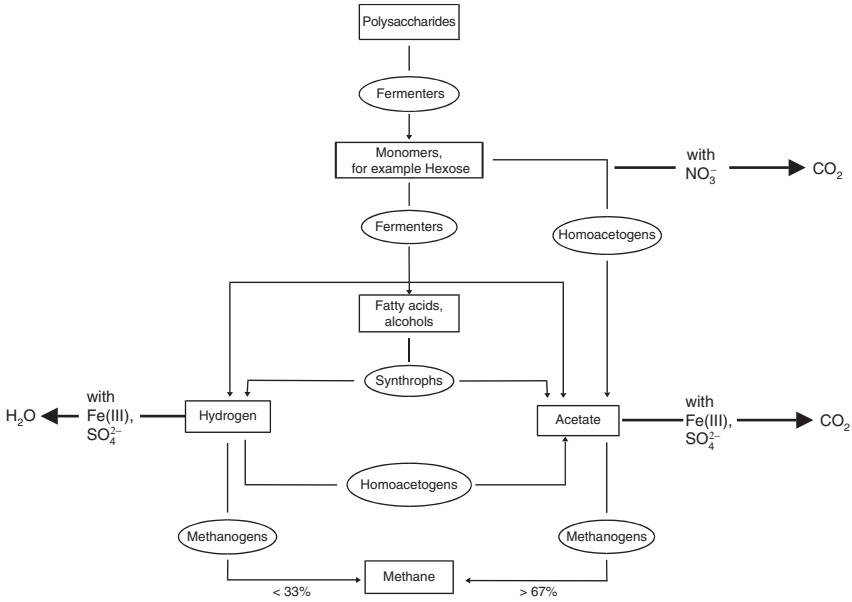


Figure 1 Pathway of anaerobic degradation of organic matter (polysaccharides) to methane. Intermediates are shown in boxes, microorganisms in ovals, the thick arrows indicate diversion of the substrate flow to reduction of nitrate, sulfate, or ferric iron.

degradation is schematically shown in Fig. 1. The path of electron and carbon flow from organic matter to CO_2 and CH_4 eventually produces acetate and H_2 at a stoichiometry in which at least two-third of CH_4 production is produced from acetate and less than one-third from H_2/CO_2 (Fig. 1). In rice field soils, the pathway of CH_4 production usually operates closely to the theoretically expected ratio (Section 2.2.2). The exact contribution of acetate versus H_2 depends on the role of homoacetogenesis, which bypasses formation of H_2 in favor of acetate (Conrad, 1999).

Rice fields are structured ecosystems and contain various habitats in which methanogens and methanotrophs can occur (Fig. 2). Most conspicuous are the following habitats: (1) The bulk soil, which is generally anoxic and reduced and occupies the largest space of the ecosystem; this habitat is limited by supply of degradable organic matter and its degradation products; it is a suitable habitat for anaerobic methanogens, but not for aerobic methanotrophs. (2) Organic plant debris, such as rice straw or dead roots; this habitat is also anoxic and reduced, but is not limited in substrate; this is also a suitable habitat for methanogens. (3) Rice roots; this habitat is partially oxic, since O_2 can locally be released from roots, and furthermore is rich in organic substrate by root exudation and decay; it is a habitat in which anaerobic methanogens and aerobic methanotrophs can live. (4) The

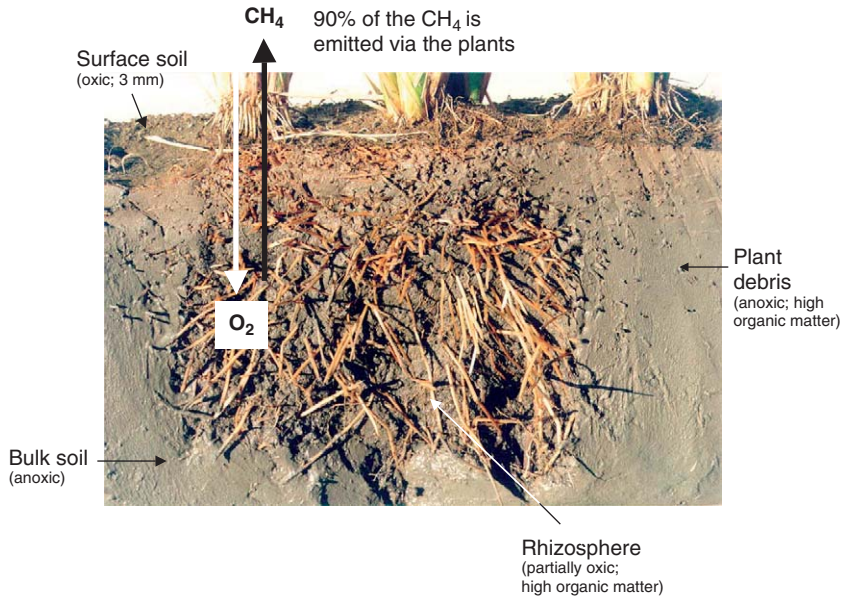


Figure 2 Cross section through a rice microcosm illustrating the major habitats of methanogenic and methanotrophic microorganisms and the exchange of CH_4 and O_2 through the gas vascular system of the rice plants. The photograph of the microcosm was provided by Dirk Rosencrantz.

shallow oxic surface layer of the flooded soil; it is a habitat suitable for aerobic methanotrophs but not for anaerobic methanogens.

In rice fields, there are three major sources of organic matter that are eventually converted to CH_4 and contribute significantly to CH_4 emission (Watanabe *et al.*, 1999). During the early season, it is mainly rice straw that is degraded to CH_4 and contributes up to 80% to CH_4 emission (Fig. 3). During this period rice plants are still small. Later in the season, however, plant photosynthesis is becoming the more important source for CH_4 production. Pulse labeling of the plants with $^{13}CO_2$ showed that up to 30% of the assimilated ^{13}C is released as $^{13}CH_4$ within 2 weeks after assimilation (Watanabe *et al.*, 1999). This rather rapid release is probably initiated by root exudation of ^{13}C-labeled photosynthates. Release of $^{13}CH_4$ after more than 2 weeks is probably derived from sloughed-off root cells or decaying roots. In total, photosynthetically derived carbon may account for more than 60% of total CH_4 emission. Finally, about 20% of total CH_4 emission is due to the degradation of soil organic carbon, that is all the organic carbon in soil that is not straw or recently produced plant carbon. The cycling of carbon in rice ecosystems has been reviewed (Kimura *et al.*, 2004).

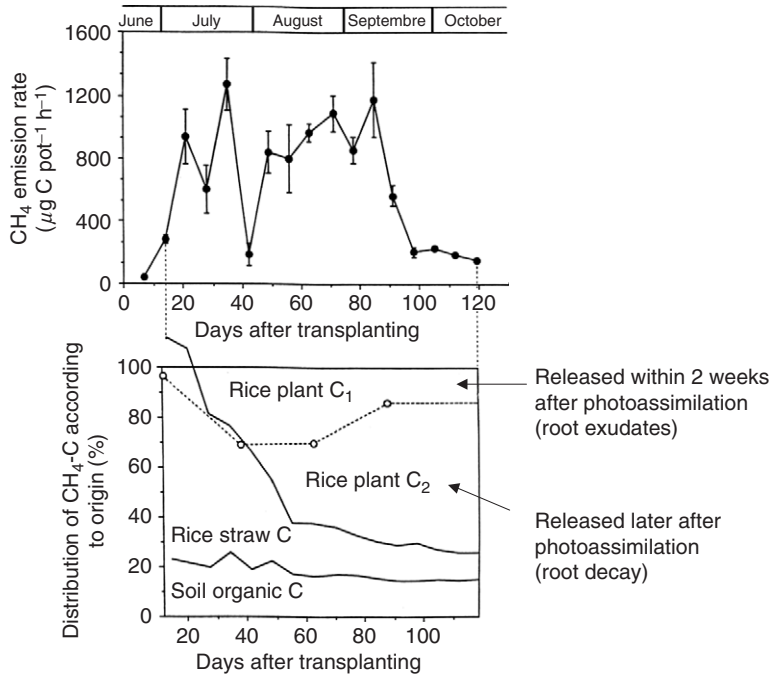


Figure 3 Emission of CH_4 from rice field microcosms and the major sources of carbon contributing to the emitted CH_4. The scheme has been adapted from Watanabe *et al.* (1999).

The methanogenic pathway of organic matter degradation (Fig. 1) mostly operates in an anoxic and reduced environment. This means that the system is not only devoid of oxygen but also of other inorganic oxidants (electron acceptors) such as nitrate, sulfate, Mn(IV), and Fe(III). In rice fields, these potential electron acceptors, Fe(III) in particular, are depleted by reduction some time after flooding, and significant CH_4 production usually does not start before this is achieved (Ponnamperuma, 1981). During the methanogenic phase, reduction of Fe(III), sulfate, and so forth usually is no longer significant in the soil. However, it may take place at the anoxic–oxic interface at the soil surface and in the partially oxic rhizosphere, where reduced Fe(II) and sulfide can be oxidized with O_2 to Fe(III) and sulfate, respectively. The production of CH_4 and the cycling of oxidants in the rice ecosystem are schematically shown in Fig. 4.

The habitats where reduced Fe and S can be oxidized are also the habitats of aerobic methanotrophic bacteria, which require O_2 for oxidation of CH_4 to CO_2. Hence, aerobic methanotrophic bacteria can potentially live only in a few microsites within the rice field (Fig. 2), that is the shallow oxic soil surface layer and the shallow oxic layer at the rice root surface (Frenzel, 2000; Groot *et al.*, 2003). Rice plants, like other aquatic plants, possess a gas vascular system (aerenchyma), which allows the diffusion of oxygen to the roots for respiration

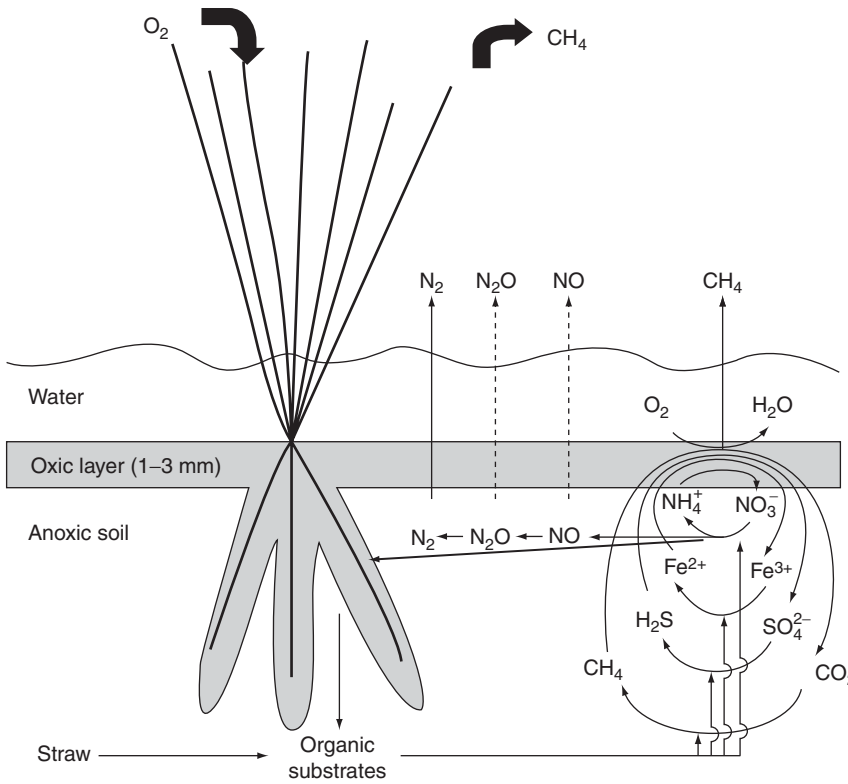


Figure 4 Reduction of CO_2, sulfate, ferric iron, and nitrate in the anoxic rice field soil and reoxidation of CH_4, sulfide, ferrous iron, and ammonium in the oxic layers at the soil water interface and the surface of rice roots. The scheme has been modified from Conrad (1996).

(Grosse *et al.*, 1996; Jackson and Armstrong, 1999). Some of the O_2 leaks from the roots and creates a very shallow and inhomogeneous oxic zone. This zone is adjacent to anoxic soil in which CH_4 concentrations can reach saturation (i.e., 1.3 mM at 25 °C) due to the permanent production of CH_4.

Vice versa, the gas vascular system of rice plants also allows the diffusion of CH_4 into the atmosphere. In fact, this is the most important path for CH_4 flux from the ecosystem into the atmosphere, provided plants have been grown (Fig. 2). Otherwise, CH_4 would accumulate in the soil until gas bubbles are formed and then released by ebullition (Kusmin *et al.*, 2006; Schütz *et al.*, 1991).

The biogeochemistry and microbiology of anaerobic processes including methanogenesis and methanotrophy have been reviewed in detail, but with focus on anoxic environments in general rather than rice fields in particular (Megonigal *et al.*, 2004). The general chemistry and biogeochemistry of submerged rice field soils has been described in a comprehensive monograph

(Kirk, 2004). A review describing the CH_4 emission rates from rice fields, important biogeochemical processes, field management, and possible mitigation options is also available (Aulakh *et al.*, 2001b). The microbiology of flooded soils has also been reviewed in detail (Conrad and Frenzel, 2002; Kimura, 2000). The present review will focus on methanogens and methanotrophs in rice field ecosystems, and describe our present knowledge of how these two groups of microorganisms are involved in the cycling of CH_4 on a microscopic scale and how these processes affect CH_4 emission on the field scale.

2. Microbial Ecology of Methanogens

2.1. Physiology and phylogeny of methanogens

The methanogenic microorganisms all belong to the phylum Euryarchaeota within the domain Archaea (Boone *et al.*, 1993; Whitman *et al.*, 2006). Within the Euryarchaeota, the methanogens are found in several orders and families (Fig. 5). All of them are characterized by the fact that they gain their energy by producing CH_4 from simple substrates such as H_2, CO, formate, and a few alcohols (isopropanol, ethanol). These substrates are oxidized to allow reduction of CO_2 to CH_4. Alternatively, CH_4 can also be produced by the reduction of the methyl groups in acetate, methanol, trimethylamine, and dimethylsulfide, part of which are oxidized to CO_2 to generate the electrons necessary for reduction of the methyl group to CH_4. Some methanogens are able to use H_2 as second substrate to reduce the methyl, for example in methanol. All reactions are thermodynamically exergonic at standard

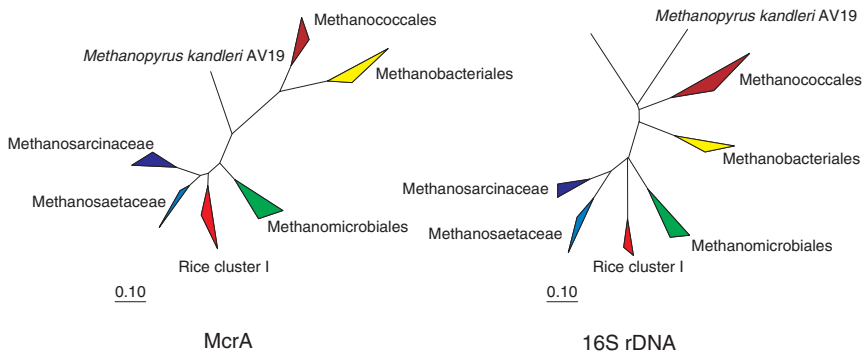


Figure 5 Comparison of the tree topologies constructed for subunit A of the methyl coenzyme M reductase (McrA) and for the 16S rRNA gene (16S rDNA) illustrating the phylogeny of methanogenic archaea. The scheme has been adapted from Conrad *et al.* (2006).

conditions, that is they may operate in nature, if substrate concentrations are sufficiently high. In rice field soils, there are two major physiological groups (guilds) of methanogens active, the acetotrophic and the hydrogenotrophic methanogens. Methanol-utilizing methanogens are also present, but methanol does not contribute significantly to total CH_4 production (Conrad and Claus, 2005).

The acetotrophic methanogens convert acetic acid to CH_4 and CO_2:

$$CH_3COOH \rightarrow CH_4 + CO_2;\ \Delta G^\circ = -35.6\,\text{kJ mol}^{-1}$$

Members of only two genera of methanogens are able to catabolize acetate, that is *Methanosarcina* and *Methanosaeta*, which belong to the families of Methanosarcinaceae and Methanosaetaceae, respectively (Fig. 5). Acetate is catabolized by cleavage, with the carboxyl group being oxidized to CO_2 and the methyl group being reduced to CH_4. The biochemical sequence of reactions is rather complex and can be found in biochemical reviews (Shima *et al.*, 2002; Thauer, 1998). For the prupose of this review only the following aspects are noteworthy (1) The CH_4-producing reaction is catalyzed by the methyl-CoM reductase, which converts methyl-CoM (methyl-coenzyme M) and HS-HTP (*N*-7-mercaptoheptanoyl-*O*-phospho-L-threonine) to CH_4 and a heterodisulfide consisting of HS-HTP and CoM-SH. This reaction is universal to all methanogens, independently of the primary substrate. This means, CH_4 in general is generated by the activity of methyl-CoM reductase. (2) The subsequent reduction of the heterodisulfide to CoM-SH and HS-HTP is coupled to the generation of a proton motive force. This reaction is the most important one for energy conservation and is universal for all methanogens. (3) In the first step, acetate has to be converted to acetyl-coenzyme A (acetyl-CoA), which requires the expenditure of energy. Formation of acetyl-CoA occurs by two different reactions (Ferry, 1992). In *Methanosarcina* spp., acetate is first phosphorylated with ATP by an acetate kinase producing acetyl-P and ADP. Subsequently, the acetyl-P is converted by a phosphotransacetylase with CoA-SH to acetyl-CoA and phosphate. In summary, conversion of acetate to acetyl-CoA requires one energy-rich phosphate bond of ATP in *Methanosarcina* spp. In *Methanosaeta* spp., on the other hand, acetate is activated using an acetyl-CoA synthetase, which converts acetate, CoA-SH, and ATP to acetyl-CoA, AMP, and pyrophosphate. In summary, this reaction requires two energy-rich phosphate bonds of ATP. This means that *Methanosaeta* spp. use more energy for acetate activation than *Methanosarcina* spp.

The hydrogenotrophic methanogens convert CO_2 with H_2 to CH_4:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O;\ \Delta G^\circ = -131\,\text{kJ mol}^{-1}$$

This type of catabolism is found among most methanogenic taxa, including the genus *Methanosarcina* (Fig. 5). The biochemical sequence can be found in biochemical reviews (Shima *et al.*, 2002; Thauer, 1998). Briefly, H_2 is oxidized to protons and the electrons generated are used to reduce CO_2 stepwise via the oxidation states of formate (formyl-MFR, formyl-H_4MPT, methenyl-H_4MPT), formaldehyde (methylene-H_4MPT), and methanol (methyl-H_4MPT, methyl-CoM) to finally CH_4. The individual C_1-compounds are bound to the coenzymes MFR (methanofuran), H_4MPT (tetrahydromethanopterin), and HS-CoM (coenzyme M). The CH_4-generating step is catalyzed by the methyl-CoM reductase, and energy is conserved (by generation of $\Delta\mu H^+$) by the reduction of the heterodisulfide, generated during this reaction. A membrane potential ($\Delta\mu Na^+$) based on sodium gradient is generated by the methyl transferase reaction from methyl-H_4MPT to methyl-CoM (Gottschalk and Thauer, 2001). However, this membrane potential is consumed during the initial activation of CO_2 to formyl-MFR and thus does not contribute to net energy gain.

The biochemistry of methanogens has consequences for biogeochemical research. One example is the fact that methyl-CoM reductase is the key enzyme present in all methanogens and only in them. This makes the gene of this enzyme a suitable target for specifically detecting methanogens in the environment. The *mcrA* gene, coding for a subunit of the methyl-CoM reductase, was found to exhibit a congruent phylogeny to that found with the 16S rRNA gene (Fig. 5). Hence, sequence information of *mcrA* genes retrieved from the environment also gives useful phylogenetic information (Lueders *et al.*, 2001). Another example is the different activation of acetate to acetyl-CoA in *Methanosarcina* and *Methanosaeta* spp., which has consequences for the ecological niches of these acetotrophic methanogens (Section 2.2.1). It apparently also affects the stable carbon isotopic signature of the produced CH_4 (Penning *et al.*, 2006a). Energetics also seems to affect the extent of isotope fractionation during reduction of CO_2 to CH_4 in hydrogenotrophic methanogenesis. At a low-energy yield, the reaction sequence from CO_2 to CH_4 is more reversible than at a high-energy yield, thus resulting in a larger fractionation factor (Penning *et al.*, 2005; Valentine *et al.*, 2004).

2.2. Diversity, habitats, and ecological niches

2.2.1. Acetoclastic methanogens

Members of both the genus *Methanosarcina* (Asakawa *et al.*, 1995; Fetzer *et al.*, 1993; Joulian *et al.*, 1998; Rajagopal *et al.*, 1988) and the genus *Methanosaeta* (Mizukami *et al.*, 2006) have been isolated from rice field ecosystems. Reports on the detection of genes (16S rRNA or *mcrA*) of *Methanosarcina* and *Methanosaeta* in rice fields are numerous (Chin *et al.*, 1999b; Grosskopf *et al.*, 1998a; Lueders and Friedrich, 2000; Wu *et al.*, 2006). A geographic

survey of several rice fields from Italy, the Philippines, and China indicates that these two acetotrophic genera are present in all soils tested (Ramakrishnan *et al.*, 2001). They were also found in Japanese rice field soil (Watanabe *et al.*, 2006). Hence, it is likely that they are cosmopolitan in all rice field ecosystems. This conclusion is not trivial, since *Methanosarcina* spp. are often missing in methanogenic lake sediments, which are usually populated by *Methanosaeta* spp. as sole acetotrophic methanogens (Schwarz *et al.*, 2007).

The abundance of methanogens has been determined in rice field habitats by using cultivation techniques and molecular methods. Cultivation techniques, generally most probable number counting using acetate as methanogenic substrate, often gave numbers of about up to 10^4 acetate-utilizing methanogens per gram dry soil (Joulian *et al.*, 1998; Schütz *et al.*, 1989b). Similar numbers of about 10^5 acetotrophic methanogens per gram dry soil were found in rooted (upper 3 cm) and unrooted (below 3 cm depth) soil layers (Frenzel *et al.*, 1999). Higher numbers (10^5–10^6 acetotrophic methanogens per gram dry soil) were found in a Japanese rice field soil in Kyushu, in particular when treated with rice straw (Asakawa *et al.*, 1998). Molecular techniques usually give higher numbers than cultivation methods. Indeed, quantitative PCR and analysis of terminal restriction fragment length polymorphism targeting archaeal 16S rRNA genes indicated that acetoclastic methanogens are present in numbers of more than 10^6 per gram dry soil in flooded rice fields (Krüger *et al.*, 2005). Theoretical considerations based on maintenance energy requirement indicate that numbers of about 10^8 per gram dry soil may be reached, if the soil is amended with rice straw (Conrad and Klose, 2006).

Both *Methanosarcina* and *Methanosaeta* spp. are able to convert acetate to CH_4. However, *Methanosaeta* spp. invest more energy to activate the acetate to acetyl-CoA (Section 2.1). Therefore, they are able to grow at very low concentrations ($<100\ \mu M$) of acetate, while *Methanosarcina* spp. require higher acetate concentrations (Jetten *et al.*, 1992). On the other hand, *Methanosarcina* spp. can grow much faster than *Methanosaeta* spp. when acetate concentrations are sufficiently high (Jetten *et al.*, 1992). In addition, *Methanosarcina* spp. can also use H_2/CO_2, methanol, or trimethylamine as energy substrates and thus are much more versatile than *Methanosaeta* spp., which only use acetate. These physiological characteristics are reflected in the ecological niches of the acetotrophic methanogens. Thus it was found that the relative dominance of *Methanosaeta* versus *Methanosarcina* spp. in anoxic rice field soil reflects the availability of acetate with *Methanosaeta* spp. becoming more abundant whenever acetate concentrations become lower than 50 μM (Fey and Conrad, 2000; Krüger *et al.*, 2005). In contrast to bulk soil, *Methanosaeta* spp. seem to play hardly a role on rice roots (Chidthaisong *et al.*, 2002; Chin *et al.*, 2004; Hashimoto-Yasuda *et al.*, 2005; Ikenaga *et al.*, 2004) and degrading rice straw (Sugano *et al.*, 2005b; Weber *et al.*, 2001a),

where acetate can reach millimolar concentrations. These habitats within the rice ecosystem are dominated by *Methanosarcina* spp., probably since the availability of acetate is relatively high and therefore *Methanosaeta* spp. are outcompeted by *Methanosarcina* spp. (Chin *et al.*, 2004). Hence, low versus high availability of acetate seems to differentiate the ecological niches of the two different acetotrophic methanogenic genera.

Niche differentiation may also be caused by temperature, as populations of *Methanosaeta* spp. in Italian rice field soil were found to tolerate low temperatures (15 °C) at nonlimiting acetate concentrations better than *Methanosarcina* spp. (Chin *et al.*, 1999b; Chin *et al.*, 1999c; Wu *et al.*, 2001, 2002). However, the effects of temperature might be different on other populations of *Methanosaeta* and *Methanosarcina* spp. when testing rice field ecosystems other than in Italy. A further interesting feature is the relative sensitivity of *Methanosarcina* spp. against phosphate on rice roots from Italian rice fields. While *Methanosarcina* spp. from culture collections easily tolerate phosphate concentrations >50 mM (Smith and Mah, 1980), the *Methanosarcina* populations on rice roots are inhibited by phosphate >10 mM (Conrad *et al.*, 2000). Although these high phosphate concentrations are irrelevant for *in situ* conditions and do not influence methanogenesis *in situ* (Conrad and Klose, 2005), the phosphate sensitivity of *Methanosarcina* root populations is a conspicuous characteristic (Lu *et al.*, 2005) differentiating this population from *Methanosarcina* populations in other systems.

2.2.2. Hydrogenotrophic methanogens

Members of the family Methanosarcinaceae, including *Methanosarcina* spp., which are commonly found in rice field ecosystems (Section 2.2.1), are also able to utilize H_2/CO_2 as energy substrate for CH_4 production. However, hydrogenotrophic methanogens are also found among other methanogenic taxa that occur in rice field ecosystems. Members of the order Methanobacteriales, for example *Methanobacterium* and *Methanobrevibacter* spp., using H_2/CO_2 have frequently been isolated from rice field soil (Adachi, 1999; Asakawa *et al.*, 1993; Conrad *et al.*, 1989; Joulian *et al.*, 1998, 2000; Min *et al.*, 1997; Rajagopal *et al.*, 1988). Members of the order Methanomicrobiales, for example *Methanospirillum* spp. (Tonouchi, 2002) or *Methanoculleus* spp. (Dianou *et al.*, 2001; Joulian *et al.*, 1998) using H_2/CO_2, have occasionally been isolated from rice field soil. An important group of hydrogenotrophic methanogens in rice fields is the so-called Rice Cluster I (RC-I), which was first described as a novel cluster of archaeal 16S rRNA gene sequences on rice roots (Grosskopf *et al.*, 1998b). In the meantime, a methanogenic enrichment culture from rice field soil (Erkel *et al.*, 2005) was used to obtain the complete genome sequence of one member of the RC-I (Erkel *et al.*, 2006). Members of RC-I probably form a family on its own or even an order within the Euryarchaota. Just recently, a Japanese group obtained the first isolate of

RC-I (Sanae Sakai *et al.*, personal communication), so that a proper taxonomic description of members of RC-I will soon be possible.

Molecular characterization (16S rRNA and *mcrA* genes) of methanogenic populations showed that potentially hydrogenotrophic Methanosarcinaceae, Methanobacteriales,Methanomicrobiales, and RC-I are widely distributed among Chinese, Philippine, Japanese, and Italian rice fields (Grosskopf *et al.*, 1998a; Ramakrishnan *et al.*, 2001; Watanabe *et al.*, 2006; Wu *et al.*, 2006). Numbers of hydrogenotrophic methanogens are on the same order (around 10^6 per gram dry soil) as reported for acetotrophic methanogens (Asakawa *et al.*, 1998; Frenzel *et al.*, 1999; Joulian *et al.*, 1998; Krüger *et al.*, 2005). The energetic conditions of methanogens strongly depend on substrate availability. Since H_2 partial pressures in rice field soil are generally low (<10 Pa), but acetate concentrations can be high (millimolar range) when soil is supplemented with straw, energetic conditions in the soil may be superior for acetotrophic than for hydrogenotrophic methanogens, thus theoretically allowing maintenance of relatively higher numbers of acetotrophic than hydrogenotrophic methanogens (Conrad and Klose, 2006). However, this is not evident from the presently available data, which rather show similar numbers of potentially hydrogenotrophic and acetotrophic methanogens.

In rice field soil, the contribution of hydrogenotrophic methanogenesis to total CH_4 production is close to the theoretically expected ratio of a third or less (Bilek *et al.*, 1999; Conrad and Klose, 2000; Rothfuss and Conrad, 1993; Yao and Conrad, 2000b). The same is the case for methanogenically degrading rice straw (Glissmann and Conrad, 2000). Occasionally, however, contributions of hydrogenotrophic methanogenesis larger than 33% were observed in Italian rice fields (Krüger *et al.*, 2001, 2002). The reasons for these relatively large contributions are presently unclear but must be due to imbalance in the degradation path of organic matter to CH_4. Possible explanations are temporary accumulation of acetate, consumption of acetate by other processes than methanogenesis, or H_2 production processes in addition to carbohydrate fermentation. The methanogenic community on the roots of rice was found to be dominated by hydrogenotrophic methanogenesis, while the simultaneously produced acetate is released into the soil (Conrad and Klose, 1999; Lehmann-Richter *et al.*, 1999; Penning *et al.*, 2006b). This dominance is also reflected in the methanogenic populations found on rice roots, which mostly belong to the hydrogenotrophic groups of Methanomicrobiales, Methanobacteriales, and RC-I (Chin *et al.*, 2004; Grosskopf *et al.*, 1998b; Hashimoto-Yasuda *et al.*, 2005; Ikenaga *et al.*, 2004), but Methanosarcinaceae, which can potentially utilize acetate, were also found (Chin *et al.*, 2004).

The question arises why the rice root community consists of so many different groups of hydrogenotrophic methanogens, although they all catalyze the same reaction. Although the reasons are not completely clear, one

important factor seems to be the availability of H_2. When roots were incubated under a H_2 atmosphere, populations of Methanosarcinaceae and Methanobacteriales incorporated $^{13}CO_2$ into their DNA, but when roots were incubated under N_2, so that only low amounts of H_2 were produced by fermenting bacteria, $^{13}CO_2$ was mainly incorporated into the DNA of RC-I methanogens (Lu *et al.*, 2005). Hence, the ecological niches of members of the RC-I methanogens seem to include utilization of low H_2 concentrations. Further ecological niches for members of the RC-I methanogens possibly are a moderately thermophilic lifestyle (Section 2.3.5), the tolerance of oxic conditions (Section 2.3.6), and adaptation to the acidic conditions found in peat (Conrad *et al.*, 2006).

The ecological niches of the other hydrogenotrophic methanogens present on the rice roots are less clear. The experiments by Lu *et al.* (2005) indicate that Methanosarcinaceae and Methanobacteriales may become active when H_2 concentrations are relatively high. However, it is unclear when this would happen under *in situ* conditions. This study of Lu *et al.* (2005) also indicates that Methanobacteriales in contrast to Methanosarcinaceae tolerate high phosphate concentrations. Although this is a niche differentiation, it is unlikely that it has relevance for *in situ* conditions (Conrad and Klose, 2005).

2.2.3. Microorganisms supplying methanogenic substrates

The microorganisms supplying the methanogenic substrates H_2 and acetate are the fermenting (primary and secondary fermentation) microorganisms and the homoacetogenic microorganisms depicted in Fig. 1. Most of the fermenters are members of the domain Bacteria, but some members of the Eukarya (protozoa, fungi) may also contribute. However, not all of the bacteria and eukarya found in rice field ecosystems are involved in the production of methanogenic substrates, since methanogenic degradation processes in the soil system are not operating for the entire year, but only during the period when the soil is flooded and then, only during the methanogenic phase after Fe(III) has been reduced. Hence, microorganisms respiring organic matter with O_2, nitrate, sulfate, and ferric iron also contribute, and may form functionally and taxonomically diverse communities by themselves. The other complexity arises from the diversity of energy substrates, mostly organic matter, but also reduced compounds like H_2, CH_4, NH_4^+, H_2S, Fe(II), and so on (Fig. 4). Most of the degradable organic matters are eventually derived from the plants, that is consisting predominantly of carbohydrates (cellulose, hemicellulose), aliphatic (fatty acids, amino acids), and aromatic (lignin, amino acids) compounds.

Our knowledge about the diversity of microorganisms in rice field soil is based on molecular studies characterizing the patterns of microbial phospholipid fatty acids (PLFA) or 16S rRNA genes. After early studies (Bai *et al.*, 2000; Bossio and Scow, 1998; Reichardt *et al.*, 1997), the

diversity of microbes in the different habitats of the rice field ecosystem has mainly been studied by the group of Makoto Kimura at Nagoya University. Their PLFA data have been summarized (Kimura and Asakawa, 2006a) showing that the microbial community structures are more or less different between the various habitats, that is floodwater, percolating water, rice soils under flooded and drained conditions, rice straw placed in flooded and drained rice soil, rice straw in the composting process, and rice straw compost placed in a flooded rice field. Their molecular analyses of bacterial 16S rRNA gene diversity give a similar picture (Cahyani *et al.*, 2003; Ikenaga *et al.*, 2003; Murase *et al.*, 2005; Shibagaki-Shimizu *et al.*, 2006; Sugano *et al.*, 2005a; Tanahashi *et al.*, 2005). Determination of the vertical distribution and temporal development of the bacterial populations in rice field soil by analysis of 16S rRNA genes demonstrates that the bacterial community is not uniform and constant, but exhibits quite some dynamics, and is also different between the oxic and anoxic parts of the system (Lüdemann *et al.*, 2000; Noll *et al.*, 2005). However, all these studies are mostly descriptive and do not allow a conclusive interpretation of which functions the various microorganisms have in the ecosystem. A few studies have applied pulse labeling of the plants with $^{13}CO_2$ followed by analysis of the rhizosphere bacterial populations that incorporated ^{13}C into their PLFA or nucleic acids (Lu *et al.*, 2004a, 2006, 2007). However, although the detected bacteria can be functionally linked to plant photosynthesis and their phylogenetic position can be determined, it is unclear which reactions they are exactly catalyzing.

The functionally relevant populations of fermenting bacteria involved in the methanogenic degradation of carbohydrates have so far been determined only in rice field soil from Italy. The following approach was used. It was shown that propionate accumulates as an important fermentation product in the soil when methanogenesis is inhibited (Chin and Conrad, 1995; Glissmann and Conrad, 2000). To identify the major groups of bacteria producing the propionate, soil was diluted so that only bacteria with an abundance of 10^8–10^9 per gram soil were left. These soil dilutions were used to isolate fermenting bacteria growing on carbohydrates (cellulose, hemicellulose, pectin, or sugar mixture) and test their major fermentation product, which indeed was propionate (Chin *et al.*, 1999a). At the same time, these soil dilutions were used to analyze the bacterial 16S rRNA genes (Hengstmann *et al.*, 1999). Thus retrieved environmental 16S rRNA gene sequences and those of the isolated bacteria were similar and mainly belonged to the *Verrucomicrobia*, the *Clostridium* Cluster XIVa, and the *Cytophaga-Flavobacterium-Bacteroides* (CFB). Hence, these bacterial groups were most likely the relevant propionate producers. Less abundant bacteria ($<10^7$ per gram soil) isolated from less diluted soil, on the other hand, belonged to other phylogenetic groups and fermented carbohydrates to butyrate or ethanol instead of propionate (Chin *et al.*, 1998).

The next step, that is the further degradation of propionate, proceeds in Italian rice field soil through the succinate pathway, which is characteristic for some of the known syntrophic fermenting bacteria that convert propionate to acetate, CO_2, and H_2 (Krylova *et al.*, 1997). The relevant propionate-consuming bacteria have recently been identified in Italian rice field soil by feeding ^{13}C-labeled propionate to methanogenic soil and determining the 16S rRNA gene sequences of the bacteria that assimilated ^{13}C into ribosomal RNA. The genera *Syntrophobacter*, *Pelotomaculum*, and *Smithella* were identified (Lueders *et al.*, 2004). Syntrophic bacteria affiliated with the genus *Pelotomaculum* seem to be widely distributed in various methanogenic environments (Imachi *et al.*, 2006). Despite this progress for Italian rice field soil, similar experiments are lacking for other rice field ecosystem found in the world. It is quite possible that the important microorganisms involved in production of methanogenic substrates are different.

2.3. Microbiological explanations for macroscopic processes, that is production and emission of methane

Methane emission patterns can be quite different at different sites, seasons, management schemes, and so forth (Wassmann *et al.*, 2000b). The most important variables that control CH_4 emission include soil type, rice variety, temperature, soil redox potential (E_h), water management, and fertilization with organic carbon and nitrogen (Aulakh *et al.*, 2001b; Kimura *et al.*, 2004; Minami, 1994; Neue and Roger, 2000; Sass and Fisher, 1997; Yan *et al.*, 2005). These variables affect production, transport, and oxidation of CH_4 in the field. This knowledge, and field and laboratory data have been used for development and testing of empirical, semiempirical and process-oriented models to simulate CH_4 emission from rice fields (Cao *et al.*, 1995; Huang *et al.*, 1998; Li *et al.*, 2004; Matthews *et al.*, 2000). However, the results of these models are not yet satisfactory. One problem is that production, transport, and oxidation of CH_4 are basic processes that are by themselves quite complex and consist of a hierarchy of subprocesses, of which the ultimate ones all operate on the microscopic scale and mostly involve microorganisms. In order to find out which are the important parameters and variables for simulation of CH_4 emission, the microscopic process level has to be understood. In the following I will review the microscopic knowledge relevant for macroscopic observations focusing on methane production and methanogenic communities.

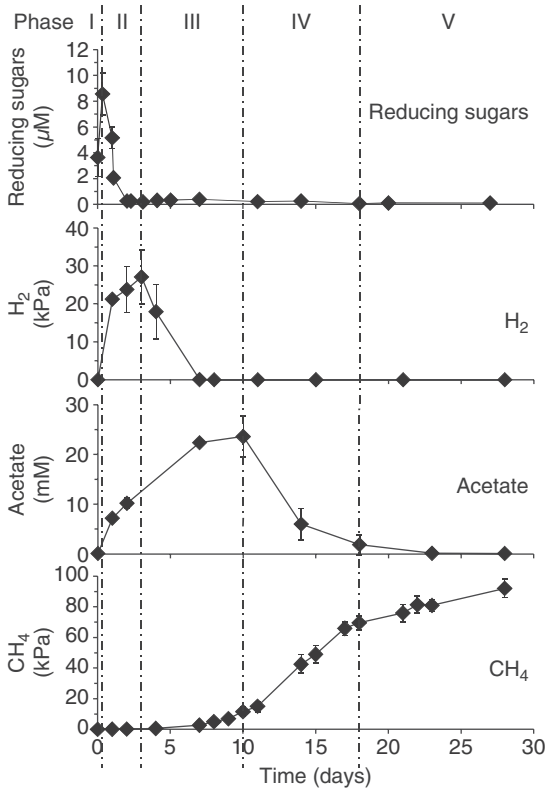
2.3.1. Sequential reduction and initiation of methanogenesis

When rice soils are flooded, production of CH_4 starts after a lag phase, then proceeds with a maximum rate and eventually slows down. These events are observed in all rice field soils, but duration and magnitude differ among the various soils (Neue *et al.*, 1994; Patrick and Reddy, 1978; Ponnamperuma, 1981;

Wassmann *et al.*, 1998; Yao *et al.*, 1999). The first phase (reduction phase) after flooding is characterized by reduction of inorganic electron acceptors, such as nitrate, sulfate, and ferric iron. During this time CH_4 production is suppressed, but subsequently develops fast, yields a maximum CH_4 production rate (methanogenic phase), and then gradually slows down (steady state phase).

Thermodynamic theory predicts that organic matter is preferentially oxidized by coupling to the reduction of nitrate, sulfate, or ferric iron rather than to CH_4 production, thus giving the thermodynamic background for the observation of a reduction phase (Ponnamperuma, 1978; Zehnder and Stumm, 1988). The sequential reduction of mostly Fe(III) and sulfate before onset of methanogenesis is often monitored by measurement of the soil redox potential (E_h) using platinum electrodes. The phase of methanogenesis is usually characterized by the E_h becoming lower than −100 mV (Wang *et al.*, 1993). However, closer inspection of the reduction phase shows that CH_4 is already produced very shortly after flooding, when the E_h is still high (Roy *et al.*, 1997). Hence, how are the processes regulated on the level of microorganisms. Figure 6 summarizes the most important events during the reduction, methanogenic and steady state phases after flooding.

Immediately after flooding, during phase I, saccharolysis of polysaccharides and fermentation starts (Glissmann and Conrad, 2002). The fermenting bacteria produce H_2, acetate, and other fermentation products from carbohydrates, for example glucose (Chidthaisong *et al.*, 1999). Thermodynamic analysis of the conditions in various rice field soils showed that hydrogenotrophic methanogenesis is usually feasible briefly after flooding (phase II) due to the relatively high partial pressures of H_2 produced by fermentation (Yao and Conrad, 1999) (Fig. 7). Indeed, hydrogenotrophic methanogens seem to be active immediately after onset of organic matter fermentation (Roy *et al.*, 1997). This observation is at the first glance surprising, since methanogens have generally been believed to require reduced conditions ($E_h < -100$ mV). However, this is obviously not generally true. Many methanogens, those isolated from soil in particular, are neither very sensitive to high redox potentials nor to exposure to O_2 (Fetzer and Conrad, 1993; Fetzer *et al.*, 1993). Genomic data show that many of them contain the genes of various O_2-detoxifying enzymes (Brioukhanov *et al.*, 2000; Shima *et al.*, 1999, 2001). In RC-I methanogens for instance, the genes coding for superoxide dismutase, superoxide reductase, catalase, desulfoferredoxin, rubrerythrin, peroxyredoxin, and H_2 oxidase are present (Erkel *et al.*, 2006). Therefore, it is not surprising that methanogens survive drainage and winter fallow of rice field soils, as they maintain virtually the same numbers per gram soil throughout the different times of the year (Asakawa and Hayano, 1995; Krüger *et al.*, 2002; Mayer and Conrad, 1990; Schütz *et al.*, 1989b). Although we do not know by which mechanism, they apparently survive dry conditions and rapidly regain activity on flooding.



	Phase of CH_4 formation				
Degradation step	I	II	III	IV	V
Hydrolysis	–	S	S	S	S
Sugar fermentation	A	–	S	S	S
$H_2 + CO_2 \rightarrow CH_4$	A	–	I S	S	S
Acetate $\rightarrow CH_4 + CO_2$	A	A	A	–	S
Reduction of SO_4^{2-}, Fe(III)	A	A	–	IS	IS

– = Not limited

A = Limited by the activity of bacteria or methanogens

S = Limited by the availability of substrate

IS = Inhibition by too low substrate concentrations

Figure 6 Phases of decomposition of organic matter to methane in anoxic rice field soil. The data of the graph are from Glissmann and Conrad (2002).

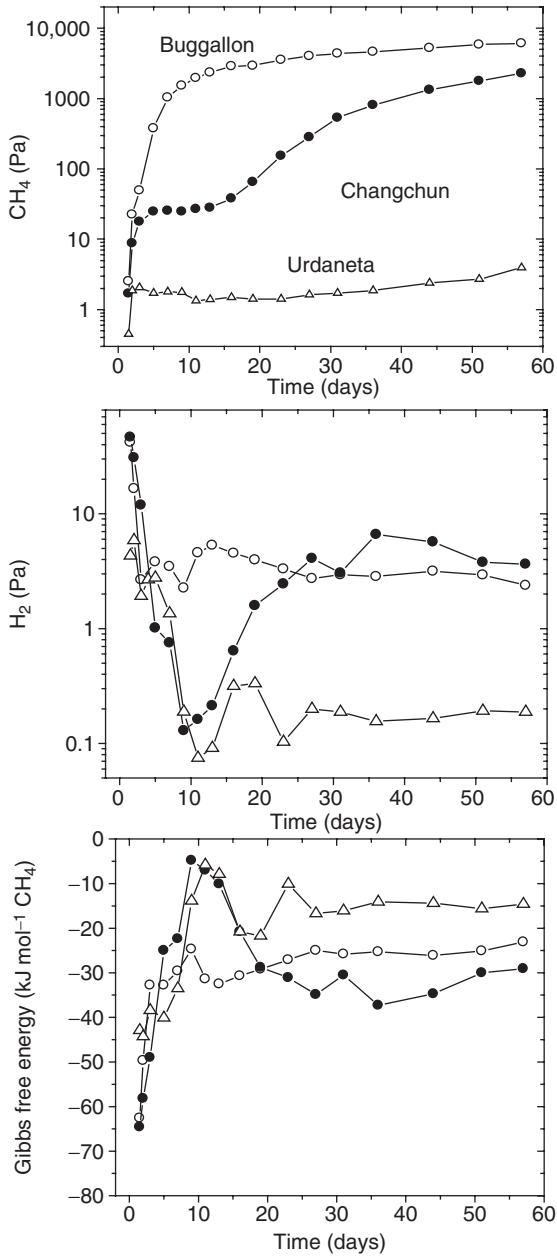


Figure 7 Temporal change of the partial pressures of CH_4 and H_2 and the Gibbs free energy (ΔG) of hydrogenotrophic methanogenesis in anoxic incubations of three different rice field soils. The ratio of available organic matter to electron acceptor (mainly sulfate and ferric iron) in the soil decreases in the order Buggallon > Changchun > Urdaneta. The data of the graph are from Yao and Conrad (1999).

They apparently regain activity faster than the sulfate and iron reducers competing for H_2. We also do not know exactly which methanogenic taxa are involved in the CH_4 production during this early phase. Molecular analysis of archaeal 16S rRNA in Italian rice field soil demonstrated the presence of Methanosarcinaceae, Methanobacteriaceae, and RC-I methanogens, all potential hydrogenotrophic methanogens, throughout the incubation (Lueders and Friedrich, 2000, 2002). Since RC-I is the most abundant group and in some experiments its abundance is decreasing with time (Conrad and Klose, 2006), RC-I methanogens are the most likely candidates for CH_4 production immediately after flooding.

Interestingly, sulfate and iron reduction, which would be thermodynamically even more feasible than methanogenesis, do not start as early as methanogenesis. The reasons are unknown, but these bacteria apparently are not yet active during phase II, while methanogens (at least some) are already active. It has been shown that sulfate reducers and iron reducers do not compete with fermenting bacteria for carbohydrates, but compete with methanogens for H_2 and acetate (Chidthaisong and Conrad, 2000). Only nitrate reducers compete with fermenting bacteria for carbohydrates, but nitrate usually is very low in rice field soil and is depleted within hours after flooding (Achtnich *et al.*, 1995; Chidthaisong and Conrad, 2000). On becoming active during phase III, sulfate and iron reducers deplete H_2 to such low concentrations that hydrogenotrophic methanogenesis is thermodynamically no longer feasible (Roy *et al.*, 1997; Yao and Conrad, 1999). This effect is especially pronounced in soils, where the content of organic matter, which allows for H_2 production, is relatively small compared to the content of reducible iron, which allows for H_2 consumption (Fig. 7). Acetotrophic sulfate reducers, mostly members of the genus *Desulfotomaculum*, often occur only as spores in the soil (Wind and Conrad, 1995).The amounts of available iron and sulfate are usually not sufficient to allow for complete depletion of acetate by sulfate and iron reducers, unless the soil is amended with additional sulfate or iron, respectively.

Despite the availability of acetate, rates of CH_4 production are nevertheless low during phase III, probably since the hydrogenotrophic methanogens are the only active ones, while the acetotrophic methanogens are not yet active during this phase. Indeed, application of molecular methods has shown that acetotrophic Methanosarinaceae increase their numbers and synthesize ribosomes for protein production resulting in increased CH_4 production in phase IV (Lueders and Friedrich, 2000, 2002). The relative increase of *Methanosarcina* spp. is reasonable because acetate concentrations are rather high. In fact, increase of numbers of *Methanosarcina* spp. is even more pronounced when rice straw is added to the soil, which results in increased fermentative acetate production (Conrad and Klose, 2006). As soon as available sulfate and ferric iron are depleted in phase IV, H_2 is no

longer consumed by sulfate and iron reducers and H_2 partial pressures rise again, so that hydrogenotrophic methanogenesis is again thermodynamically feasible and resumes (Yao and Conrad, 1999). The soil conditions then allow methanogenesis from both H_2/CO_2 and acetate, and methanogenesis becomes the sole terminal process in degradation of organic matter. In this methanogenic phase IV the rate of CH_4 production reaches a maximum. At this time, soil redox potentials (E_h) monitored with a platinum electrode have usually decreased to a low E_h of less than -100 mV.

The depletion of acetate proceeds until steady state of production and consumption of acetate is attained in phase V. The same is true for H_2 turnover for which steady state is usually reached even earlier. Soil E_h is also constantly low. The steady state phase (phase V) is in addition characterized by the production of CH_4 and CO_2 at equal rates (Yao and Conrad, 2000b), as expected theoretically from the stoichiometry of degradation of polysaccharides, for example $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$. In this phase methanogenesis is limited by the production of its substrates H_2 and acetate. The production of H_2 and acetate, on the other hand, is limited by the fermentation process, which in turn is limited by the hydrolysis of polysaccharides. Hence, in the steady state phase, CH_4 production is basically limited by the initial step of organic matter degradation (Fey and Conrad, 2003; Glissmann and Conrad, 2002), similarly as in other environments (Billen, 1982; Degens and Mopper, 1975).

In summary, the reduction phases (phases I to III) in flooded soils are the most dynamic phases with respect to microbial processes. The most important events are summarized in Fig. 6. These events are paralleled by a change in the relative contribution of hydrogenotrophic versus acetotrophic methanogenic pathways to total CH_4 production, which starts with mostly hydrogenotrophic methanogenesis in phase II, followed by mostly acetotrophic methanogenesis in phase III and IV and finally both hydrogenotrophic and acetotrophic methanogenesis at a ratio of about 20–30% to 70–80% in phase V (Conrad *et al.*, 2002; Fey *et al.*, 2004). The extent of CH_4 production is most sensitive to the relative availability of degradable organic matter versus reducible inorganic compounds, or electron donors versus electron acceptors. Hence, it is not surprising that the amount of CH_4 produced is proportional to the ratio of electron donors versus electron acceptors available in a particular soil (Yao *et al.*, 1999). These variables are more important than the soil redox potential (E_h) measured with a platinum electrode, since CH_4 production often operates at $E_h > -100$ mV (Gaunt *et al.*, 1997). Since the ratio of electron donors to electron acceptors also affects the amount of acetate that accumulates during the reduction phase (phases I–III), it also affects the maximum rate of CH_4 production in the subsequent methanogenic phase, that is phase IV (Yao *et al.*, 1999). In rice field soils, ferric iron is the quantitatively most important inorganic electron acceptor. Therefore, the degradable content of organic matter and reducible

iron are the most important soil characteristics that eventually control CH_4 production (Yao *et al.*, 1999). *Methanosarcina* spp. and RC-I methanogens seem to be the most important methanogens during the reduction phase and the subsequent methanogenic phase.

2.3.2. Effect of short-term drainage

Short-term drainage of flooded rice fields (e.g., midseason drainage) results in a strong decrease in CH_4 emission and reduces the total amount of CH_4 released from a rice field over the season (Lu *et al.*, 2000; Sass *et al.*, 1992; Yagi *et al.*, 1996; Yan *et al.*, 2005). Short-term drainage is a possible mitigation option for greenhouse gas emission (Frolking *et al.*, 2004). The immediate decrease of CH_4 emission on drainage is plausible, since O_2 can better penetrate into the soil, when it is not flooded, and thus suppress CH_4 production. However, since the suppression of CH_4 production usually persists for long time after the soil has been flooded again (Yagi *et al.*, 1996), inhibition of methanogenesis by O_2 is not a sufficient explanation for the long-term suppression of CH_4 emission. The explanation actually is that short-term drainage reverts the chemical status of the soil to the time at the beginning of flooding. The sulfate and iron in particular, which have been reduced after flooding, are apparently oxidized again during the aeration caused by short-term drainage (Ratering and Conrad, 1998; Sigren *et al.*, 1997). The thus regenerated sulfate and ferric iron allow the operation of sulfate and iron reducers, respectively. These bacteria again compete successfully with methanogens for H_2 and acetate as long as sulfate and ferric iron are available and thus suppress CH_4 production. Experiments have shown that after brief aeration of methanogenic soil, H_2 and acetate concentrations indeed decrease to such low levels that methanogenesis is no longer feasible and stay at such low levels until sulfate and ferric iron are again depleted (Ratering and Conrad, 1998; Sigren *et al.*, 1997).

Although the mechanism of short-term drainage on the microbial process level seems to be clear, it is largely unknown which microorganisms are involved in the process. The only clue comes from a field study in Italy, where an accidental short-term drainage at the beginning of the season resulted in unusually low rates of production and emission of CH_4 (Krüger *et al.*, 2001). At the same time, concentrations of ferric iron and acetate were unusually high and those of acetate unusually low, an effect expected from short-term drainage. Analysis of the methanogenic populations by targeting archaeal 16S rRNA genes showed that in the season with the relatively low acetate concentrations the ratio of *Methanosaeta* spp. versus *Methanosarcina* spp. was much higher than in the season with normal (relatively high) acetate concentrations (Krüger *et al.*, 2005). This observation is reasonable, since the ecological niches of *Methanosaeta* versus *Methanosarcina* are characterized by relatively low versus high acetate concentrations (Section 2.2.1). Nevertheless, it is unclear whether this kind of dynamic change in the populations generally

occurs after short-term drainage. *Methanosaeta* spp. have a notoriously low growth rate so that they probably can respond only slowly to environmental cues. It is probably a matter of the actual circumstances in a particular soil that define concentrations of ferric iron and acetate and thus affect methanogenic populations. Besides concentration of ferric iron, its mineral composition is an important factor affecting microbial processes. As drainage causes oxidation of ferrous iron, the freshly produced ferric iron may be easily accessible to microbes than the ferric iron that has aged over the winter fallow (Kappler and Straub, 2005). Addition of weakly crystalline ferrihydrite to rice field soil results in a more pronounced competition for available H_2 and acetate and suppression of CH_4 production than addition of more crystalline lepidocrocite, goethite, and hematite (Qu *et al.*, 2004). The observation is reasonable, since the relatively larger surface area of ferrihydrite crystals allows better accessibility to microorganisms (Roden and Zachara, 1996).

2.3.3. Effect of organic amendment

Addition of organic carbon provides electron donors to the microbial community in the rice field soil and thus enhances CH_4 production. This effect is generally seen under field conditions, when straw, compost, or manure is added (Denier van der Gon and Neue, 1995; Sass *et al.*, 1991a; Schütz *et al.*, 1989a; Yagi and Minami, 1990; Yagi *et al.*, 1997). Various studies also have shown that addition of rice straw enhances CH_4 emission much more than addition of compost or manure, coinciding with the wider range of C/N ratios in fresh straw compared to composted organic matter or manure (Agnihotri *et al.*, 1999; Chareonsilp *et al.*, 2000; Corton *et al.*, 2000; Shin *et al.*, 1996). Straw incorporated in the previous season does not enhance CH_4 emission as much as when incorporated in the same season (Yan *et al.*, 2005). Hence, CH_4 emission is apparently less stimulated if rice straw has partially been decomposed. The fate of organic matter and the cycling of carbon in rice field ecosystems has been reviewed (Kimura *et al.*, 2004). Here, I will focus on the microbial communities involved in degradation of rice straw and enhancement of CH_4 production.

The microbial colonization of straw exposed to anoxic rice field soil and its methanogenic decomposition has been studied in some detail. Rice straw is mainly composed of cellulose and hemicellulose with some minor portion (5–15%) of lignin (Tsutsuki and Ponnamperuma, 1987; Watanabe *et al.*, 1993). Microscopic investigations showed that bacteria colonize rice straw rapidly, with the easily accessible and degradable parts being colonized first (Kimura and Tun, 1999; Tun and Kimura, 2000). It is mainly hydrolytic and fermenting bacteria that colonize the straw thus explaining the rapid accumulation of acetate and various other fatty acids on addition of straw to anoxic rice soil (Glissmann and Conrad, 2000). Aromatic compounds also accumulate (Glissmann *et al.*, 2005; Tsutsuki and Ponnamperuma, 1987). However, the accumulation of the fermentation products is only transient as they are further

degraded yielding CH_4 and CO_2 as final degradation products. The bacterial communities colonizing rice straw have been characterized by targeting the 16S rRNA genes (Weber *et al.*, 2001b) or analyzing microbial PLFA patterns (Kimura and Asakawa, 2006b; Nakamura *et al.*, 2003). These studies found that *Clostridium* spp. and Gram-positive bacteria, respectively, are the major colonizing bacteria in flooded rice field soil, which is a consistent result, and was observed for the rice ecosystems in both Italy and Japan. However, analysis of 16S rRNA gene fragments retrieved from rice straw in Japanese soil showed that Alphaproteobacteria, members of the CFB group and Spirochaetes, that is all Gram-negative bacteria, were the main colonizers both under flooded and drained conditions (Sugano *et al.*, 2005a). The reason for this discrepancy to PLFA studies and results in Italian soil is unclear but may be due to the usage of different primers and PCR conditions. Interestingly, the study by Sugano *et al.* (2005a) found that the bacterial colonization was different on blade versus sheath straw and also exhibited a succession with exposure time. These two features are consistent with the microscopic investigations (Kimura and Tun, 1999; Tun and Kimura, 2000). Straw placed into drained rice fields, on the other hand, seems to be colonized mainly by Gram negative bacteria and fungi, which probably live aerobically in contrast to those found in flooded soil (Kimura and Asakawa, 2006b). Besides bacteria, the straw is also colonized by methanogenic archaea. In Italian rice soil, they mainly consist of acetotrophic Methanosarcinaceae, hydrogenotrophic Methanobacteriales, and RC-I methanogens (Conrad and Klose, 2006; Weber *et al.*, 2001a) in Japanese rice soil they mainly consist of acetotrophic Methanosarcinaceae, hydrogenotrophic Methanomicrobiales, and also RC-I methanogens (Sugano *et al.*, 2005b). However, it is unclear whether the methanogens detected on the straw are really active. This doubt comes from process studies, which showed that the microbial community on rice straw mainly supports hydrolysis and fermentation reactions, while the further conversion of fermentation products to CH_4 occurs in the soil rather than on the straw (Glissmann *et al.*, 2001). The microbial colonization pattern of straw apparently deserves more research.

The degradation of compost or manure in rice field soil has not yet been studied on a process level. However, the microbial communities have been analyzed both by targeting PLFA and 16S rRNA genes. The microbial communities were studied during the composting process of rice straw (Cahyani *et al.*, 2002, 2003, 2004a,b) and after the compost was placed into flooded rice fields and there further decomposed (Tanahashi *et al.*, 2004, 2005). Methanogens are involved in both processes. During the composting process, Methanosarcinaceae, Methanomicrobiales, and RC-I methanogens were prevalent (Cahyani *et al.*, 2004b), but thermophilic *Methanothermobacter* spp., which were found in other composting plant material (Derikx *et al.*, 1989), were not identified. The bacterial community gradually changed after putting the compost into the rice field soil. The most active bacterial groups belonged to clostridia, proteobacteria,

spirochetes, and myxobacteria (Tanahashi *et al.*, 2005). Similar data on methanogenic archaea are not yet available. So far, the microbial analysis of rice straw compost does not help explaining why addition of compost stimulates CH_4 emission to less extent than addition of uncomposted rice straw.

2.3.4. Effect of fertilization with Fe, S, and N

Addition of ferric iron can result in substantial suppression of CH_4 emission under field conditions and was recommended as an option for mitigation of CH_4 emission (Furukawa and Inubushi, 2002; Jäckel *et al.*, 2005). This effect is based on the outcompetition of methanogens by iron-reducing bacteria, which utilize the common substrates H_2 and acetate more effectively (Section 2.3.2). The suppression is especially pronounced if lower crystalline forms of iron (ferrihydrite) are applied (Jäckel *et al.*, 2005), whereas CH_4 suppression by higher crystalline forms of ferric iron (furnace slag) is dependent on the natural iron content of the soil (Furukawa and Inubushi, 2004). Since the reduction of Fe(III) to Fe(II) can accept only one electron, ferric iron would reduce the electron flow to CH_4 production only if added in large amounts. However, suppression of CH_4 production by added ferric iron is much larger than expected from the stoichiometric electron balance between iron reduction versus methanogenesis. Under field conditions, iron is probably frequently recycled into the oxidized state within the rhizosphere where O_2 is leaking from roots into the soil and thus supports iron oxidation (Begg *et al.*, 1994) (Fig. 4). It is also possible that Fe(III) has a direct inhibitory effect on methanogens. Experiments in defined microbial culture have shown that amorphous ferrihydrite can indeed inhibit methanogens directly, in particular hydrogenotrophic ones (Van Bodegom *et al.*, 2004). Some of the methanogens apparently can utilize Fe(III) as electron acceptor and reduce Fe(III) to Fe(II) instead of CO_2 to CH_4 (Bond and Lovley, 2002; Van Bodegom *et al.*, 2004).

However, little is known on the detailed biogeochemistry of the microbial processes involved in this complex process of iron cycling and methane suppression in rice field ecosystems (Ratering and Schnell, 2000, 2001). Also only few results are available from experimental microbial model systems and freshwater sediments (Roden, 2003; Roden and Wetzel, 2003; Sobolev and Roden, 2002; Weber *et al.*, 2006). The microbial populations involved in iron reduction are also largely unknown. Besides methanogens rice roots also contain (see above) potential iron-reducing bacteria such as *Geobacter* spp. and *Anaeromyxobacter* spp. (Scheid *et al.*, 2004; Treude *et al.*, 2003). However, iron oxidizers have not yet been identified on rice roots, but they occur on roots of *Typha latifolia*, another aquatic plant (Neubauer *et al.*, 2002).

Addition of sulfate to rice field soil (usually as ammonium sulfate or phosphogypsum) has a similar effect on CH_4 emission as the addition of

ferric iron (Corton *et al.*, 2000; Denier van der Gon and Neue, 1994; Lindau *et al.*, 1993, 1994). Sulfate allows sulfate reducers to outcompete methanogens for their common substrates H_2 and acetate (Sections 2.3.1 and 2.3.2). The inhibitory effect of sulfate is limited, however, if sulfate is not regenerated by oxidation of sulfide in the rhizosphere. Similarly as for iron cycling, sulfur cycling is probably taking place in the rhizosphere (Fig. 4), since sulfate concentrations increase toward the root surface (Wind and Conrad, 1997). Both sulfur-oxidizing and sulfate-reducing bacteria have been detected on rice roots in rather high diversity (Graff and Stubner, 2003; Scheid and Stubner, 2001) and it has been shown that sulfate reducers can suppress methanogenic activity in root incubations (Scheid *et al.*, 2004). However, details of the sulfur cycling and the microorganisms involved are not known.

For suppression of CH_4 emission, sulfate may be supplied as gypsum or phosphogypsum. These compounds are not very soluble. Nevertheless, the solubility constant of gypsum is $K_s = 4.2 \times 10^{-5}$ M (Stumm and Morgan, 1981), so that the equilibrium sulfate concentration is in the millimolar range. Because of the long-term supply of sufficiently high sulfate concentrations, addition of gypsum or phosphogypsum has a much stronger effect than addition of ammonium sulfate (Corton *et al.*, 2000; Lindau *et al.*, 1998). Suppression of CH_4 emission may also happen by the deposition of atmospheric sulfur. Thus, it was found that deposition of sulfate by acid rain inhibited the CH_4 emission from peat bogs (Gauci *et al.*, 2002, 2004a). This may well be a global phenomenon and affect CH_4 emission from rice fields as well (Gauci *et al.*, 2004b).

In analogy to ferric iron and sulfate, one would expect that addition of nitrate also suppresses CH_4 emission. Indeed nitrate always results in strong suppression of CH_4 production when added to methanogenic soil (Achtnich *et al.*, 1995; Klüber and Conrad, 1998a) or methanogenic rice roots (Scheid *et al.*, 2003). Suppression by nitrate is caused by competition and toxic effects. Competition occurs on two levels. First, availability of nitrate allows the consumption of glucose by nitrate reducers instead of fermenting bacteria so that the methanogenic substrates H_2 and acetate are no longer produced (Chidthaisong and Conrad, 2000). Second, the methanogenic substrate H_2 is more efficiently utilized by nitrate-reducing bacteria than by methanogenic archaea. Thus, addition of nitrate, or other reducible nitrogen compounds (nitrite, NO, N_2O) results in a decrease in the H_2 partial pressure below the thermodynamic threshold of hydrogenotrophic methanogenesis, which is then no longer possible (Achtnich *et al.*, 1995; Klüber and Conrad, 1998a). Addition of nitrate also results in oxidation of reduced sulfur and iron, so that sulfate and ferric iron are regenerated. They can then serve as electron acceptors and thus allow sulfate and iron reducers to successfully compete with methanogens for H_2 (Klüber and Conrad, 1998a). However,

a decrease of acetate concentrations was not observed on addition of nitrate, although acetotrophic methanogenesis was nevertheless inhibited (Klüber and Conrad, 1998a). Therefore, the suppressive effect on acetotrophic methanogenesis is believed to be mainly due to the production of nitrite, NO, and N_2O as intermediates of denitrification, which can be toxic for various microorganisms, including methanogens (Klüber and Conrad, 1998b; Roy and Conrad, 1999). Suppression of CH_4 production on rice roots by nitrate indeed resulted not only in inhibition of CH_4 production but also in a decrease of the population of acetotrophic Methanosarcinaceae (Scheid *et al.*, 2003).

Despite the clearly suppressive effect of nitrate addition on CH_4 production in anoxic soil, suppression of CH_4 emission by nitrate fertilization has never been observed under field conditions. One reason for the lacking suppression is probably due to the efficient uptake of nitrate by the rice plants, which scavenge nitrogen for assimilation (Fig. 4). A further reason is the fact that nitrate is reduced to gaseous nitrogen rather than ammonium, so that nitrate nitrogen is permanently lost from the ecosystem rather than recycled by oxidation in the rhizosphere. Insofar, nitrogen cycling is different from sulfur and iron cycling, where gaseous loss is small (sulfur lost as H_2S or methylated S) or absent (in case of Fe).

On the other hand, fertilization of rice fields with ammonium-based fertilizers (e.g., urea) might have some suppressive effect on CH_4 emission. Although controversial reports exist, a small suppressive effect by urea has occasionally been observed (Cai *et al.*, 1997; Dan *et al.*, 2001; Schütz *et al.*, 1989a; Wassmann *et al.*, 2000a; Xu *et al.*, 2004). Suppression of CH_4 emission by urea may be due to stimulation of CH_4 oxidation (Section 3.2.5) or suppression of CH_4 production. This suppression possibly functions via production of nitrate. Rice roots are colonized by ammonia oxidizers (*Nitrosospira* spp. and *Nitrosomonas* spp.) (Briones *et al.*, 2002, 2003), which are tightly coupled in their activity to denitrification (Arth and Frenzel, 2000; Arth *et al.*, 1998; Nicolaisen *et al.*, 2004; Reddy and Patrick, 1986; Reddy *et al.*, 1989). Hence, denitrification in the rhizosphere is fed by the supply of ammonia, while the activity of denitrifiers in turn inhibits CH_4 production by the mechanisms described above. However, it is questionable whether these processes have relevance for CH_4 production under field conditions. Since plants also use ammonium as nutrient, they compete with ammonia oxidizers (Verhagen *et al.*, 1995) and thus limit the production of nitrate and dentirification (Arth and Frenzel, 2000; Kakuda *et al.*, 1999). Addition of nitrification and urease inhibitors to rice fields usually results in suppression of CH_4 emission, indicating that coupled nitrification–denitrification in the rhizosphere ultimately benefits rather than impedes the microbial community producing CH_4 (Adhya *et al.*, 2000; Lindau *et al.*, 1993; Malla *et al.*, 2005; Xu *et al.*, 2002). The benefit of ammonium probably

operates via stimulation of plant growth and increased supply of organic substrates to the methanogenic food chain (Section 2.2.6).

2.3.5. Effect of temperature

Methane emission rates correlate with increasing temperature according to the Arrhenius equation. This can be observed over the season and on a diel basis (Sass *et al.*, 1991b; Schütz *et al.*, 1990; Wang *et al.*, 1999). The temperature effect on CH_4 emission is complex, since temperature affects virtually any biogeochemical process, including CH_4 production and CH_4 oxidation. However, the soil CH_4 production is affected not only in total but in any individual reaction involved. Thus, CH_4 production by methanogens is affected, and also the processes upstream of methanogenesis are affected, that is hydrolysis and fermentation of organic matter. As soon as steady state conditions are reached and CH_4 production is limited by hydrolysis of polysaccharides and other polymers, temperature sensitivity of hydrolysis controls CH_4 production (Fey and Conrad, 2003). However, steady state is reached rather late after flooding of soil, and under field conditions is arguably never reached. Therefore, all the individual reaction steps in the flow path of carbon from organic polymers to CH_4 (Fig. 1) may be differentially affected by temperature, if they have a different sensitivity (Q_{10}, activation energy). This may result in the transient accumulation of intermediates if temperature changes. In fact this was observed in laboratory incubations of rice soil, when temperature was shifted from 30 to 15 °C (Chin and Conrad, 1995). However, the situation is even more complex, since temperature not only affects the reactions catalyzed by the existing microbial populations but also the microbial populations themselves. Thus, temperature shifts result in pronounced changes in the composition of the methanogenic archaeal community (Chin *et al.*, 1999b; Fey and Conrad, 2000). It is likely that the communities of hydrolytic and fermenting bacteria are also changed, but this has not yet been studied. Eventually, however, temperature also affects the relative contribution of acetotrophic versus hydrogenotrophic methanogenesis to total CH_4 production (Chin and Conrad, 1995; Fey and Conrad, 2000) and the ^{13}C-stable isotopic signature of the produced CH_4 (Fey *et al.*, 2004). It is presently unclear, how temperature sensitivity of all these individual reactions finally translates into the overall CH_4 rate observed under field conditions.

An interesting observation is the existence of moderately thermophilic methanogens in rice field soil. Normally, rates of CH_4 production in rice field soil reach a maximum at about 35–40 °C. However, incubation at 40–50 °C eventually leads to proliferation of thermophilic methanogens, so that after some time, CH_4 production rates are as high at 50 °C as at 35 °C (Fey *et al.*, 2001; Yang and Chang, 1998; Yao and Conrad, 2000a). At these elevated temperatures, CH_4 production in Italian rice soil was

found to be mainly due to hydrogenotrophic methanogenesis and the methanogenic archaeal community consists almost exclusively of RC-I methanogens (Fey *et al.*, 2001). Recently it was shown that thermophilic RC-I methanogens are widely distributed in geographically different rice fields, albeit not ubiquitously. In addition it was found that members of other methanogenic taxa are also stimulated by high temperatures, indicating that thermophily is a widespread phenomenon in rice field soil (Wu *et al.*, 2006). The reason for the existence of thermophiles in rice fields that usually do not reach temperatures higher than 30 °C is unknown. Possibly, these thermophiles just form a microbial seed bank that is never expressed under field conditions, but this is not known. Also, the origin of these thermophiles is not known. One possibility is that they are introduced by addition of compost to the soil, since thermophilic methanogens are frequently detected in composting materials, RC-I methanogens in particular (Cahyani *et al.*, 2004b; Thummes *et al.*, 2007).

2.3.6. Effect of plants

Rice plants greatly affect CH_4 emission (Aulakh *et al.*, 2001b). One effect is on transport of CH_4 from the soil into the atmosphere. By forming an aerenchyma system the plants provide a passage for gases between soil and atmosphere. Most of the CH_4 emission from rice fields occurs via the rice plants. The rate of CH_4 transport depends on the CH_4 gradient and the transport capacity of the plants (Aulakh *et al.*, 2002; Hosono and Nouchi, 1997). This capacity is a function of plant morphology and thus depends on the type of rice cultivar. The transport of CH_4 through rice plants has been reviewed (Aulakh *et al.*, 2001b). However, the plants can ventilate CH_4 from the soil only after it has been produced in the soil and the rhizosphere. It was found that plants themselves can produce CH_4, possibly by photochemical decomposition of pectin and release of the methyl groups as CH_4 (Keppler *et al.*, 2006). Although this process produces only tiny amounts of CH_4, detected only by highly sensitive analytical systems, the total amounts can nevertheless be significant because of the large leaf biomass (Kirschbaum *et al.*, 2006; Parsons *et al.*, 2006). For rice fields, this process is probably of only minor importance, but has not been investigated explicitly.

Another effect of plants is root exudation that supports the methanogenic food chain in the rhizosphere and eventually leads to enhanced CH_4 emission (Aulakh *et al.*, 2001b; Conrad, 2004). More than 50% of total CH_4 emission can be due to CH_4 production from plant photosynthates (Watanabe *et al.*, 1999) (Fig. 3). Production of photosynthates and loss through root exudation is a feature that affects CH_4 production and is characteristic for a particular rice cultivar (Aulakh *et al.*, 2001a). It was found that optimization of grain yields reduces CH_4 emission probably by

reducing the loss of photosynthates through the roots and decay of plant biomass (Denier van der Gon *et al.*, 2002).

The processes involved in CH_4 production from photosynthates were elucidated by pulse labeling of rice plants, that is exposure of the plant leaves to a pulse of ^{13}C- or ^{14}C-labeled CO_2. These studies showed that pulse-labeled plants release labeled organic compounds into the rhizosphere (Dannenberg and Conrad, 1999; Lu *et al.*, 2002b, 2004b). Both dissolved organic compounds and soil organic matter become labeled, accounting on the average for 0.2% and 1–5% of the photosynthetically assimilated C, respectively. Only 3–6% of the assimilated C is released as CH_4 into the atmosphere within 16–17 days (Dannenberg and Conrad, 1999), but nevertheless accounts for >30% of the CH_4 that is emitted in total (Watanabe *et al.*, 1999). These data indicate that small changes in the carbon flow of photosynthates might produce large differences in the production of CH_4 from photosynthates. Pulse labeling of the plants also results in the labeling of microorganisms in the rhizosphere demonstrating a tight link between plant roots and soil microorganisms (Lu *et al.*, 2002a, 2004a, 2006). Interestingly, the community composition of the labeled microorganisms changes with distance to the roots, indicating that Proteobacteria and Gram-positive bacteria are more prevalent closely and distantly to the root, respectively (Lu *et al.*, 2007).

Repeated pulse labeling also allowed identification of the methanogens that incorporated labeled carbon in the rhizosphere. The RC-I methanogens were the only methanogens that assimilated ^{13}C, when plants were pulse labeled with $^{13}CO_2$ (Lu and Conrad, 2005). RC-I methanogens seem to be hydrogenotrophic methanogens (Section 2.2.2). The most likely scenario is that the plant roots provide the RC-I methanogens with an energy-rich substrate, most likely a substrate that is rapidly converted to H_2, which thus allows these methanogens to produce CH_4 and biomass from plant-derived ^{13}C. This result is consistent with the observation that the methanogenic microbial community on rice roots produces CH_4 mainly hydrogenotrophically (Section 2.2.2). It is also consistent with genomic data from RC-I methanogens (Erkel *et al.*, 2006). These data show that RC-I methanogens have a complete set of O_2-detoxifying enzymes (Section 2.3.1), which is unique among methanogens that generally have no or only a few of these enzymes. Hence, it seems that RC-I methanogens are well adapted to the partially oxic conditions in the rhizopshere. Because of the strong incorporation of labeled carbon, it is likely that RC-I methanogens are responsible for much of the CH_4 production in the rhizosphere. However, it cannot be excluded that other methanogens that are present in the rhizosphere, for example *Methanosarcina* spp., also contribute to CH_4 production although they do not specifically assimilate the labeled carbon released from the roots.

3. Microbial Ecology of Methanotrophs

3.1. Physiology and phylogeny of methanotrophs

Aerobic methanotrophic bacteria belong to the Proteobacteria. The following genera have been described and are conventionally separated into two groups (Bowman, 2006; Hanson and Hanson, 1996): Type I (belonging to the Gammaproteobacteria, family Methylococcaceae) with the genera *Methylococcus*, *Methylocaldum*, *Methylomicrobium*, *Methylosphaera*, *Methylomonas*, *Methylobacter*, *Methylosarcina*, *Methylothermus*, and *Methylohalobius*; and Type II (belonging to the Alphaproteobacteria, family Methylocystaceae) with the genera *Methylocystis*, *Methylosinus*, *Methylocella*, and *Methylocapsa*. Type I and Type II methanotrophs not only differ in phylogenetic affiliation but also in several biochemical characteristics, such as the pathway of carbon assimilation (ribulose monophosphate pathway in Type I and serine pathway in Type II) or the dominant phospholipid fatty acids (unsaturated PLFAs with 16 and 14 carbon atoms in Type I and with 18 carbon atoms in Type II).

All aerobic methanotrophs activate CH_4 with a methane monooxygenase (MMO), which requires molecular O_2 and reducing equivalents (reduced cytochrome *c* or NADH) according to the following equation, and results in the production of methanol (Dalton, 2005; Lieberman and Rosenzweig, 2004; Murrell *et al.*, 2000):

$$CH_4 + O_2 + 2[H] \rightarrow CH_3OH + H_2O$$

The oxygen atoms are recovered in the methanol and the water. The (MMO) occurs as a particulate, membrane-bound form (pMMO) and a soluble, cytoplasmic form (sMMO). With the exception of *Methylocella* spp., which have only an sMMO (Dedysh *et al.*, 2000), the pMMO is universal to all aerobic methanotrophs. The sMMO is only expressed, when copper concentrations are low (about <1 μM). The gene (*pmoA*) coding for the alpha subunit of the pMMO has been used as phylogenetic marker analogously as the 16S rRNA gene (Fig. 8). In contrast to the ribosomal RNA gene, which is universal, the *pmoA* gene has the advantage of being specific for aerobic methanotrophs (with exception of *Methylocella* spp.). However, the *pmoA* gene shares homology with the *amoA* gene coding for the ammonium monooxygenase (AMO) (Holmes *et al.*, 1995). The AMO is the key enzyme of aerobic ammonium-oxidizing nitrifiers and converts ammonia to hydroxylamine in a reaction anologously to the activiation of CH_4:

$$NH_3 + O_2 + 2[H] \rightarrow NH_2OH + H_2O$$

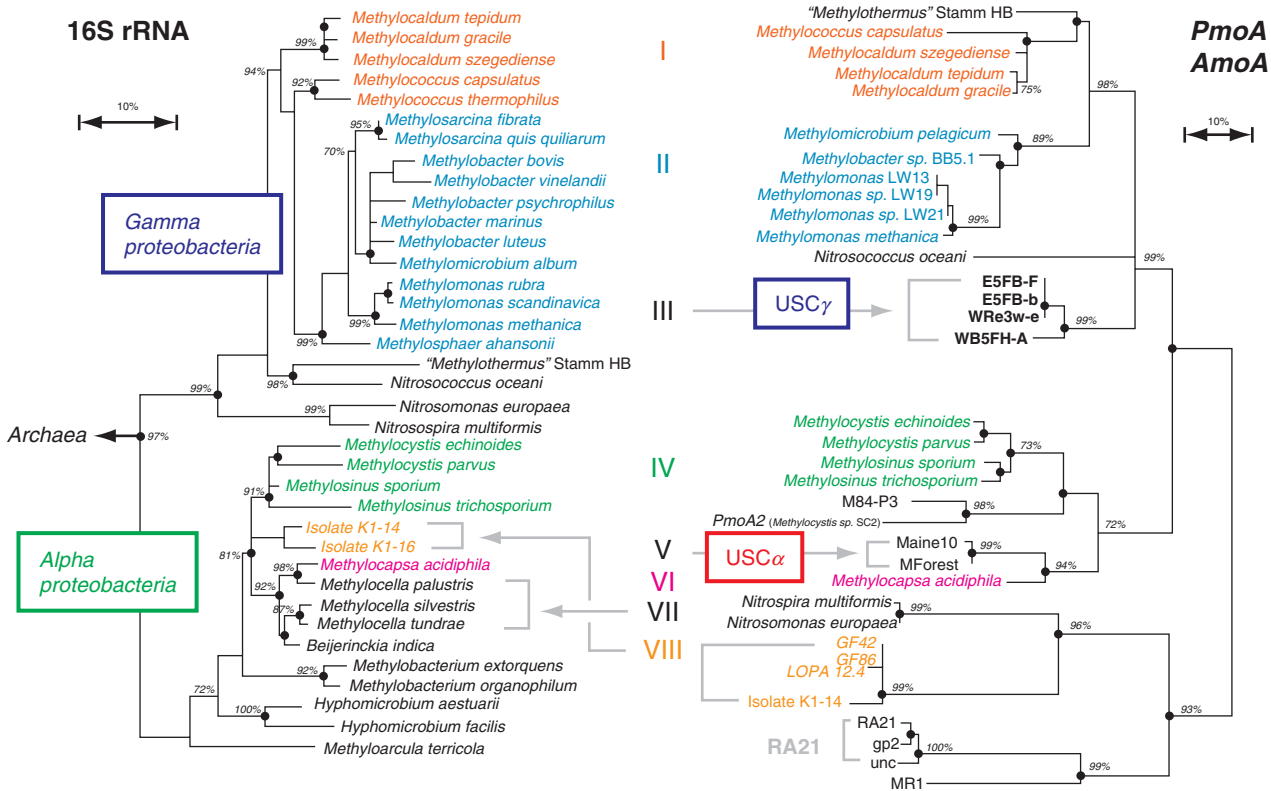


Figure 8 Comparison of the tree topologies constructed for the 16S rRNA genes and the *pmoA*/*amoA* gene products of methanotrophic bacteria including environmental sequences. The figure was provided by Stephen Kolb (PhD thesis, University of Marburg, 2003).

Assays for *pmoA* usually also detect *amoA*. This is not necessarily a disadvantage, since the AMO of ammonium-oxidizing nitrifiers (in soil mostly affiliated with the Betaproteobacteria) has also the capacity to oxidize CH_4 to methanol, albeit at a low cell-specific rate (Bedard and Knowles, 1989).

The search for methanotrophs in the environment by molecularly targeting the *pmoA* gene resulted in the discovery of *pmoA* sequence clusters for which cultivated representatives do not yet exist (Holmes *et al.*, 1999; Knief *et al.*, 2003; Kolb *et al.*, 2005) (Fig. 8). These novel *pmoA* sequences have so far only been detected in aerated upland soils, but not in flooded rice field soils. These sequence clusters, which are dubbed USCα, USCγ, Cluster I, and so on, are believed to be responsible for the uptake of CH_4 from the atmosphere (Dunfield, 2007). Consumption of low atmospheric CH_4 concentrations, equivalent to nanomolar concentrations in the soil aqueous phase, requires a higher affinity than consumption of the millimolar CH_4 concentrations emerging in the anoxic soil of rice fields. The sequencing of *pmoA* recently resulted in the discovery that *Crenothrix polyspora*, which has been known as an uncultured filamentous bacterium in water treatment plants, is actually a methanotroph of the Gammaproteobacteria (Stoecker *et al.*, 2006).

After formation of methanol by the MMO, the further dissimilation pathway is shared in methanotrophic and methylotrophic bacteria. Methylotrophs, which oxidize various C_1-compounds to CO_2, cover a much broader range of taxa than the methanotrophs (Lidstrom, 1992). They may be characterized by targeting the gene (*mxaF*) coding for the methanol dehydrogenase. This gene has also occasionally been assayed for characterizing the populations of the methanotrophs in rice field soil (Dubey *et al.*, 2003; Henckel *et al.*, 1999), but it is not specific to this group.

Anaerobic methanotrophs also exist, but none of them has yet been isolated. They mostly occur in marine sediments within syntrophic microbial consortia, and oxidize CH_4 to CO_2 by using sulfate as electron acceptor (Reeburgh, 2003). Consortia oxidizing CH_4 anaerobically with nitrate have been discovered in an anaerobic sewage digestor (Raghoebarsing *et al.*, 2006). The anaerobic methanotrophs belong to the domain Archaea. They are characterized by the sequences of their 16S rRNA and *mcrA* genes, which form the so-called ANME clusters clustering within or next to the methanogenic order of Methansarcinales (Boetius *et al.*, 2000; Hinrichs *et al.*, 1999; Orphan *et al.*, 2001, 2002; Schleper *et al.*, 2005). The mechanism of CH_4 activation is probably a reversal of the methyl-CoM reductase (Krüger *et al.*, 2003). These ANME clusters are frequently found in marine environments, but have not yet been detected in a rice field soil. Process studies indicate that anaerobic CH_4 oxidation, possibly coupled to reduction of ferric iron, may occur in the deeper strata of a rice field (Miura *et al.*, 1992; Murase and Kimura, 1994a, 1994b). However, these

early experiments have not been followed up later on. In the following I will focus on aerobic CH_4 oxidation.

3.2. Diversity, habitats, and ecological niches of aerobic methanotrophs

3.2.1. Niche differentiation

In general, little is known about niche differentiation among the different groups of methanotrophs, perhaps with exception of the thermophilic (*Methylothermus*) and halophilic (*Methylohalobius*) genera, which only occur in such extreme environments. Several hypotheses have been raised for ecological differences among Type I and Type II methanotrophs. For example, it has been hypothesized that Type I methanotrophs prefer relatively low CH_4 and high O_2 concentrations, while Type II methanotrophs conversely prefer relatively high CH_4 and low O_2 concetrations (Amaral and Knowles, 1995). Test of this hypothesis using Italian rice field soil showed that Type I in contrast to Type II methanotrophs indeed prefer relatively low CH_4 concentrations, but show no preference for high versus low O_2 concentrations (Henckel *et al.*, 2000). Furthermore, it was proposed that nitrogen availability would affect the methanotrophic populations, as Type II methanotrophs are N_2 fixers while Type I are not (Hanson and Hanson, 1996). This hypothesis was confirmed by competition experiments using defined methanotrophic strains (Graham *et al.*, 1993), and is consistent with the observation that ammonium fertilization seems to stimulate Type I more than Type II methanotrophs in the rice rhizosphere (Bodelier *et al.*, 2000b). However, N_2-fixing genes also occur among Type I methanotrophs (Auman *et al.*, 2001) and thus there is no biochemical basis for the general validity of this hypothesis. In summary, we have not yet a theoretical understanding how the different methanotrophic genera differ ecologically.

Until recently, it was believed that methanotrophs are obligate methylotrophic bacteria, that is cannot use carbon compounds with a carbon–carbon bond. However, this is obviously not true, since it has been shown that *Methylocella* spp. are able to use acetate as sole source for energy and carbon and actually prefer this compound over CH_4 (Dedysh *et al.*, 2005). Therefore, mixotrophic and heterotrophic growth have to be considered as possible ecological niches for methanotrophs in addition to methylotrophic growth.

Hence, likely effectors that may form different ecological niches are concentrations of acetate, CH_4, O_2; availability of nitrogen and copper, pH and temperature. These factors do influence the capacity of CH_4 oxidation in rice field soil (and other soils) (Bender and Conrad, 1995), but it is unknown how they operate on the microbiological scale. In summary, we may expect quite some diversity with respect to ecological

niches, which is not quite anticipated from the relative similarity in the physiology of the many different methanotrophic taxa. In the following I will review the diversity and physiology of methanotrophs in the major habitats of rice field soil (Fig. 2) and under different management.

3.2.2. Bulk rice field soil

Since the first report on aerobic methanotrophs in rice field soil (DeBont *et al.*, 1978), they have been detected in all rice field soils tested. Most probable number counts are usually on the order of 10^4–10^7 bacteria per gram soil (Dubey and Singh, 2001; Eller *et al.*, 2005; Gilbert and Frenzel, 1995; Joulian *et al.*, 1997; Watanabe *et al.*, 1995). Although the titers of methanotrophs are about an order of magnitude higher in the rhizosphere (Section 3.2.3), the bulk soil (Fig. 2) is the largest reservoir of the methanotrophic biomass in the rice field ecosystem (Eller and Frenzel, 2001; Eller *et al.*, 2005). However, since methanotrophs require O_2 for the oxidation of CH_4, they must be in an inactive state when the bulk soil is flooded. They most probably survive the anoxic conditions as a seed bank until the field is drained and O_2 becomes available again. This conclusion is consistent with the observation that most probable number counts are about one order of magnitude higher in nonirrigated versus irrigated rice fields (Dubey and Singh, 2001). Methanotrophs are able to survive periods of CH_4 or O_2 deficiency (Knief and Dunfield, 2005; Roslev and King, 1994; Schnell and King, 1995). Survival ability contributes to niche differentiation of soil methanotrophs. However, it is not quite clear by which taxa and mechanisms the survival is achieved.

The composition of the methanotrophic community in rice field soil has been determined by molecular techniques targeting 16S rRNA and *pmoA* genes (Eller and Frenzel, 2001; Eller *et al.*, 2005; Henckel *et al.*, 1999, 2001; Hoffmann *et al.*, 2002) or determining PLFA profiles (Bai *et al.*, 2000; Macalady *et al.*, 2002). Interestingly, the *pmoA* clusters (e.g., USCα) that are frequently found in upland soils (e.g., forests) have so far not been detected in the rice field ecosystem. Instead, the well-described genera of both Type I and Type II methanotrophs are detected, including *Methylobacter*, *Methylomicrobium*, *Methyolococcus*, *Methylomonas*, *Methylocaldum*, *Methylosinus*, and *Methylocystis*. Members of these genera are found in rice field soils from China, the Philippines, and Italy (Hoffmann *et al.*, 2002). However, it is unknown what the ecological niches of these different methanotrophs are.

Although the niche preferences of methanotrophs are still unclear, circumstantial evidence based on 16S rRNA analyses indicates that the community of Type II methanotrophs in Italian rice field soil may be rather stable throughout the season, while that of Type I methanotrophs changes more dynamically (Eller and Frenzel, 2001). Analysis of PLFA patterns in California rice fields, on the other hand, indicates that Type II methanotrophs correlate more with

growth of rice plants than Type I methanotrophs (Macalady *et al.*, 2002). In summary, there is a large diversity of methanotrophs in rice field soil, but little is known about the ecology of the different genera.

3.2.3. Soil surface

In contrast to the anoxic bulk soil, the soil surface layers provide a suitable habitat for activity and proliferation of aerobic methanotrophs. This habitat is a shallow (<3 mm deep) layer, where O_2 and CH_4 gradients overlap (Gilbert and Frenzel, 1998). Nevertheless, CH_4 oxidation in this shallow layer effectively scavenges >80% of the diffusive CH_4 flux from the soil into the overlying water (Conrad and Rothfuss, 1991). The surface layers of rice field soils are similar in structure to the experimental agar gradient system studied by Amaral and Knowles (1995), who have found that the zonation is Type II methanotrophs on top of Type I methanotrophs according to their preferences for CH_4 and O_2 concentrations (Section 3.2.1). Experiments on cores of Italian rice field soil showed that the CH_4 oxidation in the surface layer is inhibited by ammonium fertilization. Another study showed that Type II methanotrophs in Italian rice field soil are inhibited by ammonium (Mohanty *et al.*, 2006). Hence, it is possible that Type II might be the prevalent methanotrophs in the surface soil layer. Molecular analyses have recorded the occurrence of both Type I and Type II methanotrophs in the soil surface layer (Henckel *et al.*, 2001), but have not yet analyzed which of them account for the observed CH_4 oxidation activity. *amoA* sequences in Japanese surface soil show the presence of *Nitrosomonas* spp. and *Nitrospira* spp. of the AMO Cluster I (Bowatte *et al.*, 2006), but their contribution to CH_4 oxidation is doubtful (Section 3.2.5).

Drainage of the rice soil results in extension of the zone of CH_4 oxidation, which then progresses from the surface into deeper layers (Henckel *et al.*, 2001). This progression is accompanied with a change in the methanotrophic community at these depths, with Type I methanotrophs being the most dynamically changing group (Henckel *et al.*, 2001). Eventually, drainage yields an aerated soil, which can harbor a relatively larger number of methanotrophic bacteria than the submerged soil does (Dubey and Singh, 2001).

3.2.4. Rice roots

The rice roots with their partially oxic zones also provide suitable habitats for aerobic methanotrophic bacteria (DeBont *et al.*, 1978). Indeed numbers of methanotrophs are usually higher in the rhizosphere than in the bulk soil (Fig. 2), and the surface of the roots is also colonized (Dubey and Singh, 2001; Eller *et al.*, 2005; Gilbert and Frenzel, 1995). Methanotrophs can even invade the root cortex (Gilbert *et al.*, 1998). Although the total methanotrophic biomass on the roots is much smaller than that in the soil, it consists of methanotrophs, which are not dormant (Section 3.2.2) but immediately

active (Eller and Frenzel, 2001) and attenuate the CH_4 flux through the plants into the atmosphere (Bosse and Frenzel, 1997). Similar as at the soil surface, the methanotrophs on the roots operate at the lower end of a CH_4 concentration gradient, which extends from the soil toward the root surface (Gilbert and Frenzel, 1998) (Fig. 9). Oxygen penetrates only a short distance (<1 mm) beyond the root surface into the soil (Revsbech *et al.*, 1999). Theoretical considerations suggest that O_2 may be limiting for CH_4 oxidation (Van Bodegom *et al.*, 2001b). On the other hand, manipulation of the O_2 content in the atmosphere has indicated that CH_4 oxidation on the roots is limited by CH_4 rather than O_2 (Denier van der Gon and Neue, 1996). Concentrations of O_2 in the rhizosphere are highly variable over a wide concentration range (Gilbert and Frenzel, 1998). Therefore, both concepts are possibly true depending on location, plant variety, and physiological status. With respect to O_2 availability, it is important to which extent other processes compete with methanotrophs for O_2, such as respiration by heterotrophic microorganisms or O_2 consumption by nitrification, sulfide oxidation, or iron oxidation (Van Bodegom *et al.*, 2001a, b) (Fig. 4). However, details on the interaction between the different aerobic microorganisms on rice roots are not known.

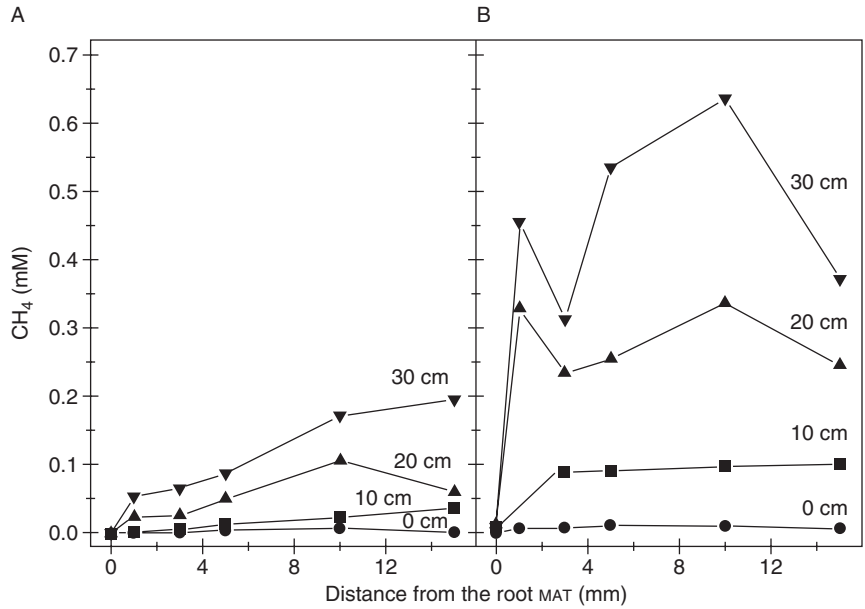


Figure 9 Concentrations of CH_4 in the porewater of (A) a three-week old, and (B) a six-week old rice microcosm. The different symbols indicate different soil depth. The figure has been adapted from Gilbert and Frenzel (1998).

The extent to which methanotrophs on rice roots attenuate the flux of CH_4 into the atmosphere is also unclear. Since CH_4 from the rice fields is predominantly emitted by transport through the rice plants and only very little through the surface soil layers (Schütz *et al.*, 1989b), CH_4 oxidation in the rhizosphere is an important process controlling the flux of CH_4 into the atmosphere. Therefore, rhizospheric CH_4 oxidation is considered in process-based flux models (Arah and Kirk, 2000; Van Bodegom *et al.*, 2001c). Over the season, the contribution of plant-mediated transport and CH_4 oxidation in the rhizosphere seem to develop in parallel (Schütz *et al.*, 1989b). Depending on the technique used, estimates of rhizospheric CH_4 oxidation range between 0% and 94%, by which the CH_4 flux is attenuated (reviewed by Groot *et al.*, 2003). It is not quite clear which factors control the attenuation process, but local O_2 concentrations are most likely among them (Van Bodegom *et al.*, 2001b). Other important factors include local CH_4 concentrations (Gilbert and Frenzel, 1998) and availability of nitrogen (Section 3.2.5). The composition of the methanotrophic community on the rice roots is probably a further important factor, which may vary with cultivar, soil, season, and management. The methanotrophic community on rice roots is highly diverse and consists of both Type I and Type II methanotrophs (Eller and Frenzel, 2001; Horz *et al.*, 2001). Type I methanotrophs seem to be stimulated by ammonium fertilizer (Bodelier *et al.*, 2000b). However, more details on the dynamics of methanotrophic populations in the root environment are not available.

3.2.5. Effect of nitrogen fertilization

Treatment of rice fields with nitrogen fertilizers was found to either increase or decrease the flux of CH_4 (Bronson *et al.*, 1997; Minami, 1995; Schütz *et al.*, 1989a). One reason could be that ammonium interacts with methanotrophs and CH_4 oxidation. This subject has been reviewed (Bodelier and Laanbroek, 2004). For example, ammonium can inhibit CH_4 oxidation. Such an inhibition has frequently been observed in nonflooded upland soils for which the sink strength for atmospheric CH_4 decreases on fertilization (King and Schnell, 1994; Mosier *et al.*, 1991; Steudler *et al.*, 1989). Inhibition of CH_4 consumption by urea was also observed in Indian rice fields under rainfed (dryland) conditions (Singh *et al.*, 1999). The mechanism of inhibition is probably based on the MMO of methanotrophs, which can also react with ammonia, so that less of the physiological substrate CH_4 is oxidized (Bedard and Knowles, 1989). In rice field soil, an inhibitory effect of ammonium has frequently been observed when measuring the CH_4 oxidation potential at elevated CH_4 concentrations (Bender and Conrad, 1995; Cai and Mosier, 2000; Dubey, 2003). Increasing ammonium concentrations intensify inhibition, which is partially reversed by increasing CH_4 concentrations (Cai and Mosier, 2000). These observations are in agreement with a competitive inhibition of the MMO by ammonia.

Inhibition of CH_4 oxidation by ammonium has also been observed in the surface soil of flooded rice fields (Conrad and Rothfuss, 1991). However, it is unclear whether this process also plays a role in the rhizosphere, where plants compete for available ammonium and thus keep its concentrations low (Verhagen *et al.*, 1995).

In rice fields, the opposite effect, that is stimulation of CH_4 oxidation by ammonium, has often been observed (Dan *et al.*, 2001; Krüger and Frenzel, 2003; Singh *et al.*, 1998b; Xu *et al.*, 2004). Like any other organisms, methanotrophic bacteria require nitrogen as a nutrient for biomass formation. Nitrogen is usually limiting in planted rice fields. Lack of sufficient nitrogen may result in inactivation or dormancy of methanotrophs, which is overcome by addition of fertilizer (Bodelier *et al.*, 2000a, 2000b). It is interesting that ammonium-based fertilizers seem to especially stimulate the Type I methanotrophs present in the rhizosphere of rice (Bodelier *et al.*, 2000b). In bulk soil (both rice and forest soil), nitrogen fertilizer also seems to stimulate Type I methanotrophs, while Type II methanotrophs are inhibited (Mohanty *et al.*, 2006). These results indicate that nitrogen fertilization has a differential effect on CH_4 oxidation, which is dependent on the resident methanotrophic populations and how they react on nitrogen addition. This means that both inhibition and stimulation are theoretically possible, but depend on the availability (competition by plant uptake) and the community composition of the methanotrophs. The conclusion that the community composition of methanotrophs is important for the behavior of the soil with respect to CH_4 oxidation is also consistent with the following observation of Chan and Parkin (2001). These authors found that the relatively low CH_4 oxidation rates of soils oxidizing CH_4 at ambient atmospheric concentrations were negatively correlated with the nitrogen content of the soil, thus indicating an adverse effect of the nitrogen status on methanotrophic activity (Fig. 10). Periodically flooded soils, on the other hand, which oxidized CH_4 at elevated CH_4 concentrations, exhibited relatively high oxidation rates, which were positively correlated to the nitrogen status of the soil (Fig. 10). Unfortunately, nothing is known about the methanotrophic bacterial communities in these soils. However, if we assume that the different availability of CH_4, O_2, and nitrogen in a particular soil translates into a different composition of the methanotrophic community, it is reasonable to assume that these different methanotrophs react differently on changes in the availability of their substrates and nutrients, that is, on fertilization. Treatment of soils with either ammonium or CH_4 can result in stimulation or inhibition of growth and activity of methanotrophs and nitrifiers (Bender and Conrad, 1994).

In analogy to the unphysiological reaction of methanotrophic MMO with ammonia instead of CH_4, the nitrifier AMO can unphysiologically react with CH_4 instead of ammonia (Bedard and Knowles, 1989; Bender and Conrad, 1994). Hence, nitrifiers may actively oxidize CH_4 to methanol

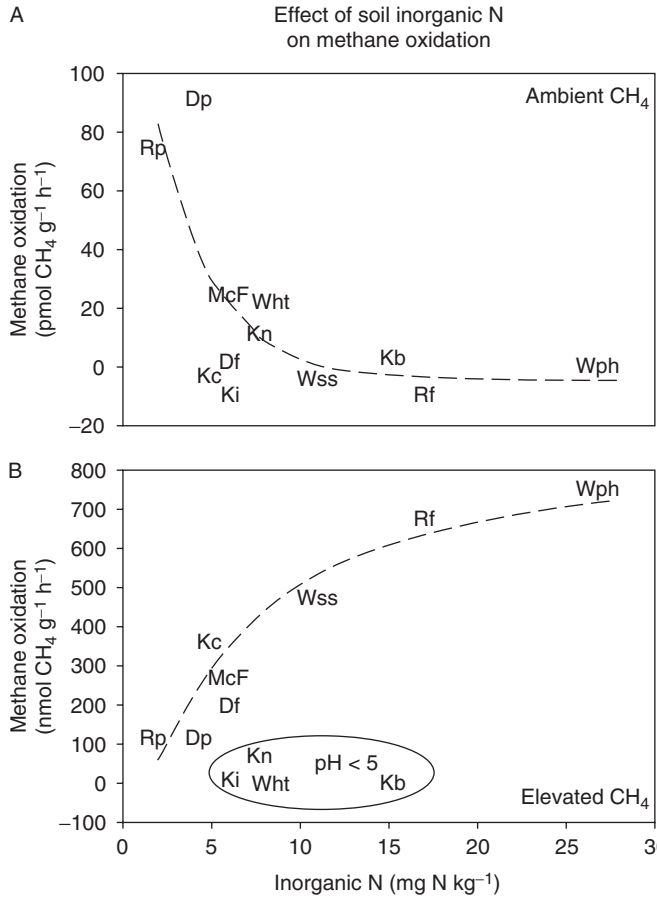


Figure 10 Methane oxidation rates as function of inorganic nitrogen concentration in different soils (labeled with letters). Methane oxidation was assayed (A) under ambient headspace CH_4 concentrations and (B) elevated headspace CH_4 concentrations. The figure has been adapted from Chan and Parkin (2001).

if they are numerous enough to compensate for the relatively low cell-specific CH_4 oxidation activity. It has indeed been observed that nitrifiers can become important for uptake of atmospheric CH_4, when agricultural upland fields are fertilized with nitrogen so that the methanotrophs are inhibited (Castro *et al.*, 1994). In rice field soil, on the other hand, nitrifiers seem not to be actively involved in CH_4 oxidation, which is exclusively catalyzed by methanotrophs (Bodelier and Frenzel, 1999). Quite in contrast, the methanotrophs compared to nitrifers seem to be strongly involved in ammonium oxidation (Bodelier and Frenzel, 1999). The negligible role of nitrifiers in CH_4 oxidation is also probably a matter of the CH_4 concentrations, which are high in rice fields, but low in upland soils,

which oxidize the CH_4 in the ambient air. Hence rice fields require a much larger capacity for CH_4 oxidation, which probably cannot be met by the unphysiological reaction of the nitrifier AMO.

3.2.6. Oxidation of atmospheric methane

It should be noted that concentrations of atmospheric CH_4 are extremely low, corresponding to about 2.4 nM in the aqueous phase. This concentration is about six orders of magnitude lower than the maximum CH_4 concentration (about 1.3 mM) in flooded rice field soil. Aerated upland soils are a significant sink for atmospheric CH_4 (Dunfield, 2007). Indian rice fields managed by dryland agriculture (rainfed conditions) also act mostly as a sink for atmospheric CH_4 (Singh *et al.*, 1998a, 1999). This is not so clear for irrigated rice agriculture. Flooded rice fields are drained at the end of the season and then are similar to an upland soil. Indeed, irrigated rice fields in the Indian Ganges plain were a source for atmospheric CH_4, but turned into a sink during the subsequent wheat crop and fallow period (Singh *et al.*, 1996). On the other hand, soil sampled from drained Japanese rice paddies in January decreased the ambient CH_4 concentration within 1 day only by a small amount of about 0.1 ppmv (calculated from the data), which is barely significant (Thurlow *et al.*, 1995). Chinese paddy soil also hardly oxidized CH_4 at ambient concentrations (1.8 ppmv), but could oxidize CH_4 at concentrations >10 ppmv (Yan and Cai, 1997). Italian rice soil apparently has the potential for oxidation of ambient CH_4 concentrations, but the CH_4 oxidation activity became inactive on drainage faster than the CH_4 production activity so that the drained soil still acted as a small source rather than a sink for atmospheric CH_4 (Jäckel *et al.*, 2001). A similar behavior has been observed in Chinese rice field soils, where CH_4 oxidation potentials were high when the soil was kept wet during the intercropping period, but decreased when the soil was kept dry (Xu *et al.*, 2003).

In summary, there seem to be two contrasting situations among rice fields. Rice fields that are frequently drained such as in rainfed and dryland rice agriculture can act as sink for atmospheric CH_4 if aerated. This situation seems to be encountered in India, where this type of rice management is widespread. The methanotrophs in these soils apparently are active enough to oxidize atmospheric CH_4. Irrigated rice fields, on the other hand, apparently do not act as a sink for atmospheric CH_4. Although these soils apparently contain methanotrophs that are able to oxidize atmospheric CH_4, they lose their activity rapidly when the soil dries up. Interestingly, Philippine rice soil managed under rainfed conditions also did not act as a net sink for atmospheric CH_4 even during the dry season planted with upland crops (Abao *et al.*, 2000). Hence, it may not only be the management but a regional difference that affects the soil behavior. The most likely explanation is that the methanotrophic communities in Indian soils are different from those in other rice-growing countries, but this is unclear,

since the only geographic overview did not include soils from India (Hoffmann *et al.*, 2002). Unfortunately, there are also few CH_4 flux data under field conditions that illustrate the situation after drainage and harvest of irrigated rice fields. However, these few show only an increased CH_4 emission on drainage, being due to the release of CH_4 bubbles entrapped in the flooded soil, but do not show any net uptake of CH_4 from the atmosphere (Denier van der Gon *et al.*, 1996; Wassmann *et al.*, 1994).

4. Mitigation of Methane Emission from Rice Fields

Rice fields are flooded to grow rice with the highest possible yield, in order to meet the increasing demand for food. Therefore, any technique used for mitigation of CH_4 emission must not compromise food production. The knowledge of the microbial processes involved in CH_4 production and emission helps to devise the optimal mitigation strategies. Many studies discuss this problem and offer mitigation options (Majumdar, 2003; Mosier *et al.*, 1998; Wassmann *et al.*, 2000a; Yagi *et al.*, 1997). The following management techniques are usually listed: water management, nutrient management, and crop management.

Water management is probably the most efficient mitigation option. Mid-season drainage or frequent intermittent drainage generally results in a drastic reduction of CH_4 production and emission. The microbiological background explaining the efficiency of the drainage strategy has been discussed in this review (Section 2.2.2). The most important argument against frequent drainage is that this might increase production and emission of N_2O (Bronson *et al.*, 1997; Cai *et al.*, 1997), which has a tenfold higher global warming potential than CH_4. However, N_2O is usually only emitted for short periods and management can be adjusted such that N_2O emission does not compromise the mitigation of CH_4 emission in terms of global warming potential (Nishimura *et al.*, 2004; Towprayoon *et al.*, 2005; Yang *et al.*, 2003; Yue *et al.*, 2005; Zheng *et al.*, 2000). Proper management of nitrogen fertilization is in particular important.

The most important nutrient management is the amendment of soil with organic matter, which results in a drastic increase of CH_4 production and emission. The microbiological basis of this management technique has been discussed (Section 2.2.3). Mitigation of CH_4 emission can be achieved when as little organic matter is added to the soil as possible. When organic matter has to be added at all, composted organic matter is preferable over uncomposted material, such as straw.

Another nutrient management is addition of oxidants, such as ferric iron or sulfate to the soil, which suppress CH_4 production and reduce emission

to quite some extent (Section 2.2.4). However, these mitigation strategies have to be carefully checked against possible adverse effects on the crop yield.

Addition of nitrogen fertilizer may result in reduced CH_4 emission rates, as CH_4 oxidation in the rhizosphere is enhanced (Section 3.2.5). The mitigation effect seems to be relatively short-lived, as the plants rapidly scavenge the nitrogen, but field experiments are scarce and equivocal (Dan *et al.*, 2001; Krüger and Frenzel, 2003; Singh *et al.*, 1998b; Xu *et al.*, 2004). Nitrogen fertilization has the potential to increase the production and emission of N_2O. However, very little N_2O is normally produced when the rice field is kept flooded (Bronson *et al.*, 1997; Cai *et al.*, 1997).

Crop management has also some promise as possible mitigation option for CH_4 emission. However, this option must be handled carefully as it affects the crop directly. Fortunately, it seems that increasing the grain yield may go in parallel with reducing the CH_4 emission (Denier van der Gon *et al.*, 2002). The beneficial effect is probably due to decreased production of root exudates that drive methanogenesis (Section 2.2.6). However, the plant variety also affects the extent of gas ventilation between soil and atmosphere and thus affects the availability of O_2 in the rhizosphere and thus the oxidation of CH_4 by methanotrophs (Section 3.2.4). Virtually no data exist on the effect of plant variety on methanogenic and methanotrophic microbial communities in the rhizosphere and on the roots. This knowledge might help to optimize the development of rice varieties with maximum grain yield and minimum support for CH_4 emission.

5. Conclusions and Outlook

The study of microbial communities has for a long time been limited by the availability of suitable methods. Hence, our knowledge of biogeochemical processes and fluxes is much more mature than our knowledge of the microbial communities that catalyze these processes. This is also true for rice field ecosystems. Nevertheless, substantial progress has been made by applying molecular techniques to the microbiota in rice fields. With respect to the populations of methanogens and methanotrophs these molecular techniques have mostly targeted 16S rRNA genes as phylogenetic marker genes and *mcrA* and *pmoA* genes as functional marker genes, respectively. The combination of molecular analysis of the microbial community and functional analysis of biogeochemical processes basically allows the assignment of function to microbial populations. In practice this can be a very difficult task if microbial communities and/or biogeochemical processes are complex. In this respect, the methanogenic and methanotrophic microbial communities in rice field soils provide a rather well-defined model system.

Both methanogens and methanotrophs catalyze chemical reactions that can be described by a stoichiometrically exact equation. The biochemistry of these processes is rather well understood. The microbes depend on these reactions as sole source for energy. They consist of monophyletic groups, and the phylogenetic trees of the 16S rRNA and functional genes (those coding for the key enzyme) are congruent. Studying these chemically well-defined processes and well-defined guilds of microorganisms clearly helps assigning structure and function within these microbial communities.

Flooded rice fields likewise are rather well-defined ecosystems and are relatively easily accessible to experimentation. Wetland soils in contrast to upland soils exhibit a macroscopic redox zonation, which describes the potential occurrence of chemical reactions. Admittedly this zonation can be rather complex around the rice roots, but it is still less complex than in soil crumbs of a forest or meadow ecosystem. Whereas such a redox zonation is also found in lake sediments or sediments of other wetland ecosystems, rice fields have the additional advantage of being managed ecosystems. This makes it possible to collect samples without worrying about sediment history. It is also possible to collect dry soil samples when the fields are drained, transport these samples to a laboratory or greenhouse and restart the rice ecosystem from scratch by flooding and planting. Such excellent experimental accessibility is not given for a natural wetland.

Therefore, it has been possible to describe processes and microorganisms involved in the production and oxidation of CH_4 relatively well, as reviewed in this article. Hence, it has been possible to describe macroscopic events, such as the temporal change of CH_4 production after flooding of the soil or the effect of fertilization, also by analyzing methanogenic or methanotrophic microbial communities. This provides some interpretation of the macroscopic events by processes on the microscopic level. Such interpretation is necessary to gain confidence in how production and oxidation of CH_4 is controlled by environmental factors, to generate appropriate process-based models and make regional and global predictions. This task is certainly not yet finished and many more data are required, in particular to better understand the processes occurring in the rhizosphere. Nevertheless, it becomes apparent that most of such data will provide a microbial interpretation of the biogeochemistry. On the other hand, these data do not necessarily provide a profound understanding of the ecology of the methanogens and methanotrophs. In other words, microbial analysis serves the understanding of biochemistry in a descriptive way, but does not help so much to understand why the microbial community is as it is. This demands a better understanding of the intrinsic ecology of the microorganisms, in particular learning more details about the ecological niches that the different microbial species occupy. Although this review has also addressed the question after the various ecological niches of the methanogens and methanotrophs, there are only very few answers. For example, we are now beginning

to understand niche differentiation (e.g., acetate concentration) between *Methanosarcina* spp. on the one hand and *Methanosaeta* spp. on the other. Nevertheless, it is highly unsatisfying that we have still no idea why we have such a large diversity of methanogens and methanotrophs in the rice field soil, although they in principle all serve the same function for the ecosystem. I am advocating the study of the rice ecosystem as a suitable model system for gaining more profound knowledge on the ecology of microorganisms in general. This may be of additional value to that describing the microbiology of rice fields as a dominant source for food and an important ecosystem for the global change of atmospheric greenhouse gases.

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