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Minireview

Methane, microbes and models: fundamental understanding of the soil methane cycle for future predictions

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Summary

Methane is an important greenhouse gas and microbes in the environment play major roles in both global methane emissions and terrestrial sinks. However, a full mechanistic understanding of the response of the methane cycle to global change is lacking. Recent studies suggest that a number of biological and environmental processes can influence the net flux of methane from soils to the atmosphere but the magnitude and direction of their impact are still debated. Here, we synthesize recent knowledge on soil microbial and biogeochemical process and the impacts of climate change factors on the soil methane cycle. We focus on (i) identification of the source and magnitude of methane flux and the global factors that may change the flux rate and magnitude in the future, (ii) the microbial communities responsible for methane production and terrestrial sinks, and (iii) how they will respond to future climatic scenarios and the consequences for feedback responses at a global scale. We also identify the research gaps in each of the topics identified above, provide evidence which can be used to demonstrate microbial regulation of methane cycle and suggest that incorporation of microbial data from emerging -omic technologies could be harnessed to increase the predictive power of simulation models.

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Introduction: methane in the context of global warming

Human activities have been identified as a major cause of increased emission of the three main greenhouse gases (GHGs) [carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)] since the beginning of the industrial era (IPCC, 2007; Eusufzai *et al.*, 2010). CH_4 constitutes the second most significant greenhouse gas after CO_2, and it accounts for up to 20–30% of global warming effect (IPCC, 2007). The Earth's atmosphere had a stable, relatively constant CH_4 concentration of 0.7 p.p.m. (parts per million) until the 19th century when a steady increase brought concentration to 1.745 p.p.m. in 1998 (IPCC, 2007). During the late 1970s and early 1980s, the rate of increase in CH_4 concentration was as high as 1% per year but a minor slowdown started in the mid-1980s. Atmospheric CH_4 has since 2005 stabilized at a value of 1.77–1.78 p.p.m. Recently, a slight imbalance towards an annual increase of about 0.1% per year of CH_4 emission has been calculated (IPCC, 2007). Despite a short lifetime in the atmosphere of approximately 8 years, CH_4 is 25 times more potent than CO_2 as a GHG, over a 100-year horizon and on a mass basis due to its higher efficiency in trapping radiation (Shindell *et al.*, 2009). An increase in the atmospheric concentration of CH_4 to 2.55 p.p.m. and a lifetime in the atmosphere of 8.4 years is predicted by 2050, based on current rates of CH_4 emissions (Lelieveld *et al.*, 1998). Consequently, it is essential to understand the processes responsible for the sources and sinks of CH_4 in order to better control anthropogenic emissions.

Methane is produced by microbial communities as a part of anaerobic respiration. Microbial CH_4 production (methanogenesis) is performed by a specific group of *Archaea,* the methanogens, and occurs in anoxic environments as a consequence of fermentation, the anaerobic

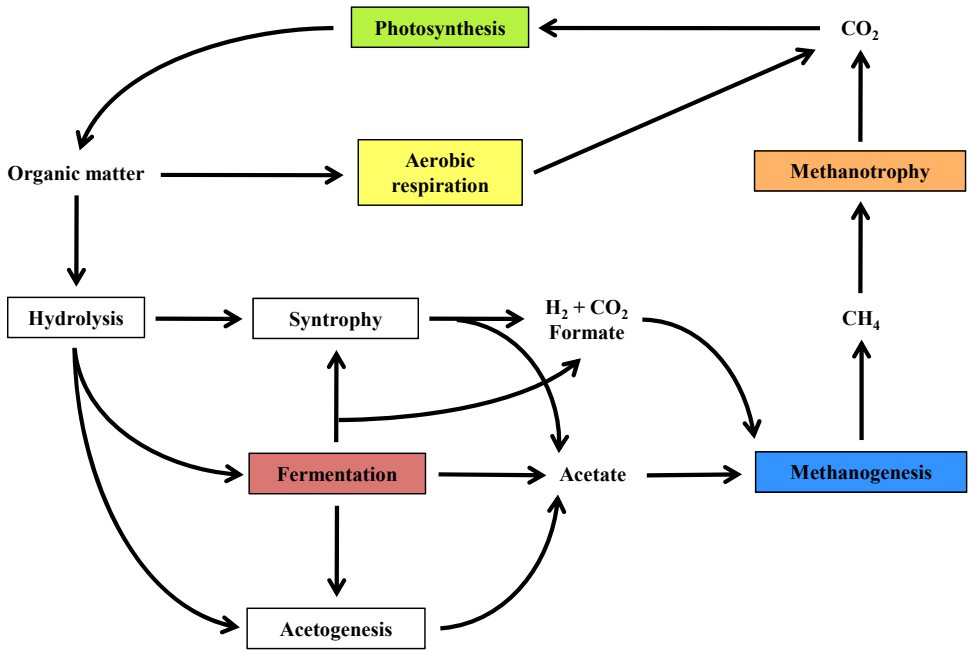

Fig. 1. Global methane cycle in nature and its linkage with carbon cycle. The five main compartments of C processing are indicated by the coloured boxes. Arrows indicate the substrates either produced by a process or for which other process intermediates are produced.

degradation of organic matter. Archaeal methanogens only constitute the last step of fermentation and rely on the presence of a larger bacterial consortium including hydrolytic, fermenting, syntrophic and acetogenic bacteria (Cicerone and Oremland, 1988) (Fig. 1). In contrast, microbial CH_4 consumption (methanotrophy) is mainly achieved by a unique group of *Proteobacteria* called methanotrophs. The majority of methanotrophs are aerobic organisms. Thus, CH_4 oxidation usually takes place at anoxic/oxic boundaries such as sediments, and in soils in particular, where methanotrophs play an important role in attenuating CH_4 emissions to the atmosphere subsequent to production of methane in the deeper anoxic environments (Conrad, 2009) (Fig. 1).

Methane is naturally emitted from sources such as wetlands, oceans, sediments, plants, termites and geological sources (e.g. gas seepage, hydrates). Wetlands represent the largest source, accounting for 62% of the (natural) CH_4 budget (Fig. 2). Anthropogenic sources include rice agriculture, livestock, landfills and waste treatment, biomass burning and fossil fuel extraction and consumption. During the pre-industrial era, natural sources represented over 90% of total emission whereas man-made emissions dominate present-day CH_4 budgets, accounting for 63% of the total global emissions (Conrad, 2009), and occur mostly in the northern hemisphere (Lelieveld, 2006). Most of the natural CH_4 sources are of microbial origin (Conrad, 1996). In the troposphere, chemical removal of CH_4 from the atmosphere represents 88% of the total sink. It is performed through the photochemical oxidation of CH_4 initiated by the reaction with •OH radicals (Cicerone and Oremland, 1988). The loss of CH_4 in the stratosphere accounts for about 7% of the sink. Finally, CH_4 is also eliminated from the atmosphere by

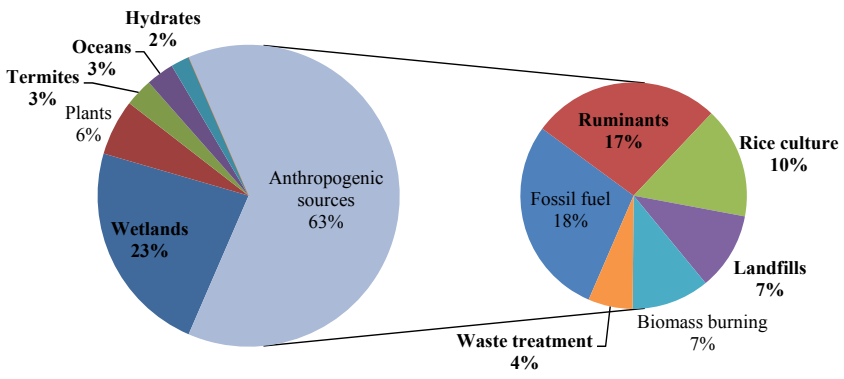

Fig. 2. Global sources of atmospheric CH_4. The left side of the larger figure chart represents natural CH_4 sources, whereas its right side gives an estimate of anthropogenic activity-related sources as detailed on the right. In bold is microbial-related production. Adapted from IPCC (2001) and Conrad (2009).

uptake in upland soils due to microbial oxidation by methanotrophs (estimated at 5% of the microbial sink) (Conrad, 2009).

Knowledge of global CH_4 sources and sinks is essential to devise efficient mitigation strategies to combat global warning. Reducing anthropogenic emissions of CH_4 can have an effect on the global CH_4 budget. This was a primary goal in establishing the Kyoto Protocol of 1997 (UNFCCC, 1998). However, the anthropogenic impact on CH_4 consumption is limited because the terrestrial sink contribution to the global budget is small (only 5–7%), nonetheless, importantly this process will ultimately determine whether the Earth, as a whole ecosystem, is a source or sink for CH_4. It is estimated that more than 50–90% of the CH_4 produced belowground is oxidized before reaching the atmosphere (Reeburgh, 2003; Kvenvolden and Rogers, 2005). This is in conjunction with the sink of atmospheric CH_4 occurring mostly in upland soils, and especially in temperate forests (Ojima *et al*., 1993; Le Mer and Roger, 2001). Therefore, it is important to better understand the mechanisms involved in CH_4 emissions from soils and CH_4 sinks in soils. This review will highlight the importance of soil microbial communities and their control of net CH_4 flux and propose reasons why they should be included in prediction models to improve the accuracy of climate change simulations.

Methane microbiology

Methane-producing Archaea *(methanogens)*

Methanogens are strictly anaerobic obligate CH_4-producing *Archaea*. Methanogenesis represents the terminal step of the anaerobic food chain, converting methanogenic substrates to CH_4 (Hedderich and Whitman, 2006). Methanogens constitute an ancient monophyletic lineage within the *Euryarcheota* phylum. They are classified into three classes, six orders, 12 families and 35 genera (Table 1). A novel lineage of methanogens, rice cluster I (RC-I), was identified from rice roots by culture-independent approaches (Großkopf *et al*., 1998; Lueders *et al*., 2001). However, RC-I members are widely distributed in other habitats around the world (Conrad *et al*., 2006). They form a distinct clade within the *Methanomicrobiales* and *Methanosarcinales* radiation based on the genetic analysis of 16S rRNA and *mcrA* (encoding methyl-coenzyme M reductase) (Großkopf *et al*., 1998; Lueders *et al*., 2001). Recently, members of RC-I were isolated in pure culture and the new *Methanocellales* order was created (Sakai *et al*., 2007; 2008; 2010; 2011). Similarly the *Methanoregulaceae* family was added to the *Methanomicrobiales* order to accommodate members of the following new genera: *Methanoregula* (Bräuer *et al*., 2011; Yashiro *et al*., 2011), *Methanosphaerula* (Cadillo-Quiroz *et al*., 2009) and *Methanolinae* (Imachi *et al*., 2008; Sakai *et al*., 2012) (Table 1).

Most methanogens are mesophiles; however, several genera of methanogens (Table 1) can be found in extreme environments such as marine geothermal sediments, hot springs and hypersaline sediments. Because methanogens cannot use complex organic compounds, they need the presence of other anaerobes in the environment to degrade these compounds into simple sugars and fatty acids then further fermented by syntrophic bacteria to form acetate, formate, hydrogen (H_2) and CO_2, which constitute the main substrates for methanogenesis (Fig. 1). Acetogens (acetate-producing bacteria) are part of this syntrophic consortium when methanogens consume H_2 and formate efficiently (Stams, 1994). Most methanogens are H_2-consumers because they need H_2 as an electron donor and consequently, they need to closely interact with H_2-producing microorganisms, and also as a way to remove reducing equivalents (Hedderich and Whitman, 2006; Stams and Plugge, 2009).

CH_4-production pathways and key enzymes. The complexity and uniqueness of methanogenesis as a form of anaerobic respiration resides in the requirement of six unusual coenzymes [ferredoxin (Fd), methanofuran (MFR), tetrahydromethanopterin (H_4MPT), coenzyme F_{420} (F_{420}), coenzyme M (CoM) and coenzyme B (CoB)]; a multistep pathway and several unique membrane-bound enzyme complexes coupled to the generation of a proton gradient driving ATP synthesis (Ferry, 2010). The three main methanogenic substrates are CO_2, acetate and methyl group containing compounds (such as methanol, methylated amines and methylated sulfides). Therefore, three distinct (hydrogenotrophic, aceticlastic and methylotrophic respectively) pathways for CH_4 production exist (Ferry, 1999; Deppenmeier, 2002) (Fig. S1).

Although the intermediates and enzymatic reactions of the three pathways are different, they share common features in the final steps of CH_4 production (Fig. S1). The hydrogenotrophic and aceticlastic pathways both result in the production of a carrier-bound methyl intermediate. The carrier protein is H_4MPT in the hydrogenotrophic pathway and tetrahydrosarcinapterin (H_4SPT), a derivative of H_4MPT, in the aceticlastic pathway. The transfer of the methyl group to CoM by a specific, membrane-bound methyltransferase (MTR), and the subsequent reduction of methyl-CoM to CH_4 by the key enzyme methyl-coenzyme M reductase (MCR) (Thauer, 1998), is common in all three pathways. The three methanogenic pathways are described in more details in *Supporting information*.

MCR consists of a dimer of three subunits, α (McrA), β (McrB) and γ (McrG), and contains a unique porphinoid nickel (Ni)-containing active site called coenzyme F_{430}

Table 1. Taxonomy of major methanogens.

Domain/kingdom/phylum	Class	Order	Family	Genus	Major CH_4 production pathway
Archaea/Archaebacteria/ Euryarchaeota	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*	*Methanobacterium*	H_2/CO_2, methylotrophic
				Methanobrevibacter	
				Methanosphaera	
			Methanothermaceae	*Methanothermobacter*	
				Methanothermus	
	Methanococci	*Methanococcales*	*Methanococcaceae*	*Methanococcus*	H_2/CO_2
				Methanothermococcus	
			Methanocaldococcaceae	*Methanocaldococcus*	
				Methanotorris	
		Methanomicrobiales	*Methanomicrobiaceae*	*Methanomicrobium*	H_2/CO_2
				Methanoculleus	
				Methanofollis	
				Methanogenium	
				Methanolacinia	
				Methanoplanus	
			Methanospirillaceae	*Methanospirillum*	
			Methanocorpusculaceae	*Methanocorpusculum*	
				Methanocalculus	
			Methanoregulaceae	*Methanoregula*	
				Methanolinae	
				Methanosphaerula	
		Methanocellales (RC-I)	*Methanocellacaea*	*Methanocella*	H_2/CO_2
				Methanosarcina	Aceticlastic, methylotrophic
				Methanococcoides	
		Methanosarcinales	*Methanosarcinaceae*	*Methanohalobium*	
				Methanohalophilus	
				Methanolobus	Methylotrophic
				Methanomethylovorans	
				Methanimicrococcus	
				Methanosalsum	
			Methanosaetaceae	*Methanosaeta*	Aceticlastic
	Methanopyri	*Methanopyrales*	*Methanopyraceae*	*Methanopyrus*	H_2/CO_2

The extremophilic/tolerant methanogens are also colour-coded: blue: extreme thermophiles (growth > 80°C); yellow: extreme halophiles (growth at 4.3 M NaCl). Adapted from Liu and Whitman (2008).

(Gunsalus and Wolfe, 1980). The enzyme's apparent molecular mass is about 300 kDa. Two distinct isoenzymes of methyl-CoM reductase were identified (Steigerwald *et al*., 1993). The second enzyme, designated MRT for methyl reductase two, has a different substrate affinity (Bonacker *et al*., 1993). MCR activity is encoded by the *mcrBDCGA* operon, while the *mrtBDGA* operon codes for the MRT (Thauer, 1998). The equivalent of the gene *mcrC* is missing in the *mrt* operon (Pihl *et al*., 1994). The products of the genes *mcrC* (McrC), *mcrD* (McrD) and *mrtD* (MrtD) are below 20 kDa. Their function is still unknown (Reeve *et al*., 1997).

Primary sensors and signal transduction cascades have not been elucidated. However, evidence was found for regulation by trace elements and their availability (Hedderich and Whitman, 2006). This is because many enzymes of methanogenesis contain trace metals (molybdenum, tungsten, selenium, nickel) in their active site. Availability of the substrate H_2 was found to regulate the formation of some key enzymes of methanogenesis including MRC. In *Methanothermobacter* species, the expression of the two isoenzymes of MCR is differentially regulated by H_2 availability, with isoenzyme I (MCR) predominantly expressed in H_2-limiting conditions (Bonacker *et al*., 1992; Morgan *et al*., 1997). The regulation of gene expression in methanogens is still not fully understood and needs further study.

Methane-oxidizing microbes (methanotrophs)

Methanotrophs are Gram-negative aerobic bacteria and generally only grow on CH_4 or methanol as a source of carbon (C) and energy. Methanotrophs are often found at the anoxic/oxic interface of various habitats such as geothermal reservoirs, landfills, soils, peat bogs, wetlands or aquatic environments and sediments, where they consume the CH_4 arising from methanogenesis and are thus able to lower these CH_4 emissions (Whalen *et al*., 1990; Conrad and Rothfuss, 1991; Conrad, 1996). These methanotrophs can oxidize high concentrations of CH_4 (> 100 p.p.m.), and are sometimes referred to as low-affinity methanotrophs, and many can be cultivated in the laboratory. Other methanotrophs have the ability to oxidize CH_4 at atmospheric (low) levels (~ 1.8 p.p.m.) and are known as high-affinity methanotrophs (Bender and Conrad, 1992). They have not yet been cultivated although they have been identified in upland soils using molecular and biochemical methods (Holmes *et al*., 1999; Henckel *et al*., 2000; Knief *et al*., 2003). These high-affinity methanotrophs are mainly grouped into two clades, USCα and USCγ, and are distantly related to two groups (respectively, type II and type I) of cultivable methanotrophs (Holmes *et al*., 1999; Bull *et al*., 2000; Knief *et al*., 2003; 2006; Singh and Tate, 2007). The majority of aerobic methanotrophs use CH_4 as their sole source of C and energy (obligate methanotrophs). Facultative methanotrophs also exist (see section below) and can grow on multi-carbon substrates. The majority of extant aerobic methanotrophs are neutrophiles (growing at a pH of ~ 6.0–8.0) and mesophiles (growing at 20–40°C) (Whittenbury *et al*., 1970). Nevertheless, several species of methanotrophs have been isolated from extreme ecosystems displaying high and low values of temperature, pH or salinity (Trotsenko and Khmelenina, 2002; Op den Camp *et al*., 2009) (Table 2).

To date, aerobic methanotrophs are known to have representatives in two phyla, three orders and four families. A total of 21 genera and 56 species have been identified (Table 2). Based on morphological, physiological and genetic differences (refer to *Supporting information* for more details), extant CH_4-oxidizing bacteria have traditionally been divided into two groups: type I and type II. Nowadays, they are referred to as *Gammaproteobacteria* and *Alphaproteobacteria* respectively (Table 2). Type I methanotrophs (referred to as gammaproteobacterial methanotrophs in this review) belong to the *Methylococcaceae* family in the *Gammaproteobacteria.* Type II methanotrophs (*Alphaproteobacteria*, or alphaproteobacterial methanotrophs here) are divided into two families: the *Methylocystaceae* and the *Beijerinckiaceae* (Bowman *et al*., 1993; Op den Camp *et al*., 2009). Among the *Methylococcaceae*, the genera *Methylovulum* (Iguchi *et al*., 2011) and *Methylomarinum* (Hirayama *et al*., 2013) were recently added, as well as two new *Methylomonas* species [*M. paludis* (Danilova *et al*., 2012) and *M. koyamae* (Ogiso *et al*., 2012)] (Table 2). Furthermore, two genera of filamentous methanotrophs occur: *Clonothrix* and *Crenothrix* (Stoecker *et al*., 2006; Vigliotta *et al*., 2007). Although 16S rRNA-based studies group them with gammaproteobacterial methanotrophs, *Crenothrix* shows a very divergent *pmoA* gene (encoding for a key polypeptide of pMMO) (Stoecker *et al*., 2006). Newly isolated microorganisms expanded the phylogeny of alphaproteobacterial methanotrophs with the addition of the genus *Methyloferula* (Vorobev *et al*., 2010) and a new member of the *Methylocystaceae* family, *Methylocystis bryophila* (Belova *et al*., 2013). Finally, CH_4-oxidizing bacteria of the *Verrucomicrobia* phylum were recently identified and the three species that were isolated from geothermal habitats in Italy, New Zealand and Russia (Dunfield *et al*., 2007; Pol *et al*., 2007; Islam *et al*., 2008) were classified under the *Methylacidiphilum* genus of the *Methylacidiphilales* order (Op den Camp *et al*., 2009) (Table 2). These methanotrophic *Verrucomicrobia* represent a unique group of methanotrophs capable of growing at very low pH value although they share many characteristics of methanotrophic *Proteobacteria*, especially of alphaproteobacterial methanotrophs (Hou *et al*., 2008). It

Table 2. Taxonomy of aerobic methanotrophs.

Domain: *Bacteria*/Kingdom: *Eubacteria*/Phylum: *Proteobacteria*				
Class: *Gammaproteobacteria* Order: *Methylococcales*			Class: *Alphaproteobacteria* Order: *Rhizobiales*	Phylum: *Verrucomicrobia* Class: *Verrucomicrobiae* Order: *Methylacidiphilales*
Family: *Methylococcaceae*			Family: *Methylocystaceae*	Family: *Methylacidiphilaceae*
Methylobacter	***Methylomonas***	***Methylocaldum***	***Methylocystis***	***Methylacidiphilum***
Methylobacter bovis	*Methylomonas aurantiaca*	*Methylocaldum gracile*	*Methylocystis echinoides*	*Methylacidiphilum infernorum*
Methylobacter chroococcum	*Methylomonas fodinarum*	*Methylocaldum szegediense*	*Methylocystis heyeri*	*Methylacidiphilum fumarolicum*
Methylobacter luteus	*Methylomonas methanica*	*Methylocaldum tepidum*	*Methylocystis hirsute*	*Methylacidiphilum kamchatkensis*
Methylobacter marinus	*Methylomonas rubra*		*Methylocystis methanolicus*	
Methylobacter psychrophilus	*Methylomonas scandinavica*	***Methylococcus***	*Methylocystis minimus*	
Methylobacter tundripaludum	*Methylomonas paludis*	*Methylococcus capsulatus*	*Methylocystis parvus*	
Methylobacter vinelandii	*Methylomonas koyamae*	*Methylococcus thermophilus*	*Methylocystis pyriformis*	
			Methylocystis rosea	
Methylomicrobium	***Methylosarcina***	***Methylogaea***	*Methylocystis bryophila*	
Methylomicrobium agile	*Methylosarcina fibrata*	*Methylogaea oryzae*		
Methylomicrobium album	*Methylosarcina lacus*		***Methylosinus***	
Methylomicrobium buryatense	*Methylosarcina quisquiliarum*		*Methylosinus sporium*	
Methylomicrobium pelagicum			*Methylosinus trichosporium*	
Methylohalobius	***Methylosphaera***		Family: *Beijerinckiaceae*	
Methylohalobius crimeensis	*Methylosphaera hansonii*		***Methylocapsa***	
			Methylocapsa acidiphila	
Methylosoma	***Methylovulum***		*Methylocapsa aurea*	
Methylosoma difficile	*Methylovulum miyakonense*			
			Methylocella	
Methylothermus	***Methylomarinum***		*Methylocella palustris*	
Methylothermus thermalis	*Methylomarinum vadi*		*Methylocella silvestris*	
Methylothermus subterraneus			*Methylocella tundrae*	
Clonothrix	***Crenothrix***		***Methyloferula***	
Clonothrix fusca	*Crenothrix polyspora*		*Methyloferula stellata*	

The extremophilic/tolerant methanotrophs are also colour-coded: orange: psychrophiles (growth at 5–10°C but not above 20°C); purple: haloalkaliphiles (growth at 12% NaCl and at pH of 9–11); yellow: halophiles (growth at 15% NaCl); blue: thermophiles (growth >45°C); green: acidophiles (growth at pH of 3.8–5.5); grey: thermoacidophiles (growth at 60°C and at pH of 2). Adapted from Trotsenko and Murrell (2008).

appears that verrucomicrobial methanotrophs can fix CO_2 to use as sole source of carbon while CH_4 is used for energy generation (Khadem *et al*., 2011; Sharp *et al*., 2012). More details on the biochemistry, physiology and genetics of methane oxidation by this group have recently been uncovered (Hou *et al*., 2008; Khadem *et al*., 2010; 2011; 2012a,b,c).

Anaerobic oxidation of methane (AOM) is carried out by a tight/physical association of anaerobic methanotrophic (ANME) *Archaea* and sulfate-reducing bacteria (SRB), *Desulfosarcina* and *Desulfococcus* of the *Deltaproteobacteria* class (Hinrichs *et al*., 1999; Boetius *et al*., 2000; Knittel and Boetius, 2009). AOM involves the use of sulfate as an electron acceptor to oxidize CH_4 via a process of reversed methanogenesis (Thauer and Shima, 2008). AMNE *Archaea* are close relatives of the methanogenic *Archaea* (*Methanosarcinales* and *Methanomicrobiales*) and are divided into three main clusters: ANME-1, ANME-2(a, b and c) and ANME-3 (Knittel and Boetius, 2009). Although originally thought to be obligate methanotrophs, a recent analysis suggested that the impact of the ANME-1 group on the global methane budget should be re-evaluated as these organisms were able to revert to methanogenesis in CH_4-producing sediments (Lloyd *et al*., 2011). AOM coupled to denitrification was described from enriched cultures (Raghoebarsing *et al*., 2006). In particular, the bacterium *Methylomirabilis oxyfera* is able to anaerobically reduce nitrite to form its own supply of O_2 via a new intra-aerobic pathway (Ettwig *et al*., 2009; 2010). The presence of small concentrations of O_2 (2–8%) has an overall inhibitory effect on this organism (Luesken *et al*., 2012). Genomic analysis revealed that this newly discovered anaerobic denitrifying methanotroph does not use reversed methanogenesis to oxidize CH_4 but rather the typical aerobic methanotrophic mechanism and despite the apparent absence of intracytoplasmic membrane system (Wu *et al*., 2011; 2012). Apart from using sulfate and nitrite as electron acceptors, AOM was also been found to be dependent on manganese and iron in marine environments (Beal *et al*., 2009).

Oxidation of CH_4. Aerobic methane oxidation is catalysed by methane monooxygenase (MMO). MMO exists in two forms: a soluble form (sMMO) and a membrane-bound, or particulate, form (pMMO) (Prior and Dalton, 1985; Lipscomb, 1994; Hakemian and Rosenzweig, 2007; Semrau *et al*., 2010) (detailed information in *Supporting information*). Although these two enzymes catalyse the same reaction, their mechanism and origin are very different (Holmes *et al*., 1995; Semrau *et al*., 2010). The oxidation of CH_4 into methanol is followed by the formation of formaldehyde by the enzyme methanol dehydrogenase (MDH). Formaldehyde constitutes the central point of methanotroph metabolism. Two pathways were identified for its assimilation and these were originally used by Whittenbury and colleagues (1970) to separate methanotrophs into type I and type II (Fig. S2). Gammaproteobacterial (type I) methanotrophs use the ribulose monophosphate (RuMP) pathway whereas alphaproteobacterial (type II) methanotrophs assimilate formaldehyde through the serine pathway (Lawrence and Quayle, 1970; Trotsenko and Murrell, 2008). Only a portion (~ 50%) of carbon at the oxidation level of formaldehyde is assimilated into cellular biomass (Prior and Dalton, 1985). The remainder is converted into formate and ultimately into CO_2 to generate reducing power for the initial oxidation of CH_4 and for biosynthetic reactions and energy generation (Fig. S2). A third pathway for carbon assimilation in some methanotrophs is the fixation of CO_2 via the Calvin–Benson–Bassham (CBB) cycle by verrucomicrobial methanotrophs (Op den Camp *et al*., 2009) (Fig. S2). Other methanotrophs (*Methylocaldum*, *Methylococcus*, *Methylogaea*) also possess RubisCO activity (see Table 3) but it is not known if, or how, these bacteria use the CBB pathway in nature. Other differences between proteobacterial and verrucomicrobial methanotrophs are detailed in Table 3 and can be found in *Supporting information*.

Both forms of methane monooxygenase (sMMO and pMMO) require O_2 but they use a different electron donor/acceptor systems (Lieberman and Rosenzweig, 2004; Hakemian and Rosenzweig, 2007; Semrau *et al*., 2010) (Fig. S2). sMMO is a very versatile enzyme capable of oxidizing a wide range of alkanes, aliphatics and aromatic compounds (Colby *et al*., 1977). pMMO is present in all methanotrophs, except in *Methylocella* and *Methyloferula* spp. (Dedysh *et al*., 2000; Dunfield *et al*., 2003; Vorobev *et al*., 2010). A number of methanotrophs of the genera *Methylomonas*, *Methylomicrobium*, *Methylovulum*, *Methylococcus*, *Methylocystis* and *Methylosinus* contain both pMMO and sMMO (Table 3).

Methylocella and *Methyloferula* species use sMMO to grow on methane and are facultative methanotrophs, also growing on multi-carbon compounds such as acetate, pyruvate, succinate, malate and ethanol (Dedysh *et al*., 2005; Rahman *et al*., 2011). The evolutionary reasons behind obligate methanotrophy are still unknown (Chung *et al*., 1988; Dedysh *et al*., 2005; Theisen and Murrell, 2005; Trotsenko and Murrell, 2008; Chen *et al*., 2010a; Semrau *et al*., 2011). Recently, other facultative alphaproteobacterial methanotrophs have been isolated, *Methylocapsa aurea* (Dunfield *et al*., 2010), *M. bryophila* and *Methylocystis* strain SB2 (Im and Semrau, 2011; Yoon *et al*., 2011; Belova *et al*., 2013). Like *Methylocella*, these microorganisms are mostly found in acidic soils and all belong to the *Beijerinckiaceae* family (Table 3). A detailed inventory of the known facultative methanotrophs, the

Table 3. Some characteristics of known aerobic methanotrophs.

Gram-negative, aerobic	Type I		Type II		*Verrucomicrobia*
Phylum/class	*Gammaproteobacteria*		*Alphaproteobacteria*		*Verrucomicrobia*
Genera	*Methylobacter, Methylomicrobium, Methylomonas, Methylosarcina, Methylosphaera, Methylohalobius, Methylothermus, Methylosoma, Methylomarinum, Methylovulum, Clonothrix, Crenothrix*	*Methylocaldum, Methylococcus, Methylogaea*	*Methylocystis, Methylosinus*	*Methylocapsa, Methylocella, Methyloferula*	*Methylacidiphilum*
Methanotrophy	Obligate		Obligate	Obligate Facultative[a]	Obligate
ICM formation	Bundles of vesicular disks distributed throughout the cell and perpendicular to cell periphery		*Methylocystaceae* – membrane stacks packed in parallel to cell periphery *Methylocapsa* – membrane vesicles packed parallel to only one side of cell membrane *Methylocella* – vesicular membranes connected to ICM		No but carboxysome-like structures or vesicular membranes
Formaldehyde assimilation	RuMP pathway	RuMP pathway; low levels of enzymes of the serine pathway	Serine pathway	Serine pathway RuMP pathway[b]	A variant of the serine pathway
MMO activity	pMMO; sMMO[c]		pMMO; sMMO	pMMO[d]; sMMO[e]	pMMO
pmoA genotype affiliation associated with oxidation of atmospheric CH_4[f]	USCγ; cluster 1, cluster 3 *Methylococcaceae*		*Methylocystaceae*	USCα; cluster 5 *Beijerinckiaceae*	*Methylacidiphilaceae*
Major PLFA biomarkers	14:0; 16:0; 16:1ω7c; 16 ω5t 18:1ω7[g]	16:0; 16:1ω7c	18:1ω8c 18:2 ω6c,12c; 18:2 ω7c,12c[h]	18:1ω7c	i14:0; a15:0; 18:0
Nitrogen fixation	*Methylosphaera, Methylosoma, Methylogaea* and *Methylococcus* only		Yes		Yes
RubisCO activity	No	Yes	*Methyloferula* only		Yes
G+C (mol%)	43–63	57–65	62–67	56–63	41–46

a. Only in *Methylocella* and *Methylocapsa* aurea.
b. Only in *Methyloferula*.
c. Only in *Methylomonas*, *Methylomicrobium*, *Methylovulum* and *Methylococcus*.
d. Not in *Methylocella* or *Methyloferula*.
e. Not in *Methylocapsa*.
f. Kolb (2009).
g. Only in *Methylohalobius*.
h. Bodelier and colleagues (2009).
Data were compiled from Op den Camp and colleagues (2009), Semrau and colleagues (2010) and recent literature.
Abbreviations: RuMP, Ribulose monophosphate; pMMO, particulate methane monooxygenase; sMMO, soluble MMO; USC, upland soil cluster; ICM, intracytoplasmic membrane.

different pathways for acetate assimilation and their ecological applications (particularly in the field of bioremediation) has been recently reviewed (Im and Semrau, 2011; Semrau *et al*., 2011; Yoon *et al*., 2011).

Although there is some understanding of the genetics of methanotrophs (operon structure, gene copy number and variation, regulation by copper – see *Supporting information* for more details), a number of important knowledge gaps still exist in the microbiology, biochemistry and genetics of methane oxidation (Box 1).

Box 1. Research gaps in methane microbiology.

(1) Production of CH_4 has been observed in marine systems where methanogens could not be detected by molecular methods (Biddle *et al*., 2008; Valentine, 2011). However, recent work has confirmed that methanogens are omnipresent and become more active when the environmental conditions become favourable (Conrad, 2009). Furthermore, it is believed that some methanogens are aero-tolerant (Conrad, 2009) and can thrive in the pelagic zone and oxic water column of many water bodies (Reeburgh, 2007; Grossart *et al*., 2011). Their identification in ecological studies, in particular in soils, will be key findings.

(2) Metagenome sequencing suggests that the vast majority of methanogens are still to be cultivated (Biddle *et al*., 2008).

(3) One of the greatest challenges for the scientific community is to isolate high-affinity methanotrophs which can oxidize CH_4 at atmospheric concentration. This is a crucial step required for understanding the phenotypes and physiological capabilities of methanotrophs.

(4) Further detailed studies on gene regulation and how methanogens and methanotrophs respond, at the molecular level, to changes in environmental conditions are needed. Additional studies are required to identify the mechanisms responsible for anaerobic methane oxidation and also how N_2O is formed under anaerobic conditions.

(5) Information on ecology and niche adaptation of methanogens and methanotrophs will provide key information to utilizing microbial traits in predictive models.

(6) Deciphering the role of facultative methanotrophs in the global budget of CH_4 is needed.

(7) Newly discovered methanotrophs outside of the *Proteobacteria* phylum (Op den Camp *et al*., 2009) highlights the increased diversity of methanotrophs. Emerging metagenomics, transcriptomics and proteomics techniques can now be used to help identify novel methanogens and methanotrophs from many different environments. The success of such approach can be achieved, for example, by combining enrichment methods, such as stable-isotope probing (SIP), with metagenomics (Chen and Murrell, 2010; Chen *et al*., 2010b). Another important technique will be the combination of fluorescence *in situ* hybridization (FISH) to stable-isotope Raman spectroscopy, or Raman-FISH (Huang *et al*., 2007; Neufeld *et al*., 2007b).

Ecology of methanotrophy and methanogenesis

Both abiotic and biotic environmental factors are considered as strong regulators of CH_4 flux (Lessard *et al*., 1994; Castro *et al*., 1995). Methanotrophic activity is lowered while methanogenesis is increased when soil moisture increases above field capacity due to a decrease in O_2 availability (Czepiel *et al*., 1995; Sitaula *et al*., 1995). The atmospheric CH_4 sink mainly occurs in upland forest soils and this function is attributed to high-affinity methanotrophs (Kolb, 2009). In temperate forest and poorly drained soils, when moisture ranged from 60% to 100% water-filled pore space (WFPS), CH_4 oxidation rates decreased (Whalen and Reeburgh, 1990; Bender and Conrad, 1995). The authors attributed this to limited O_2 availability and soil gas diffusivity, in particular because CH_4 transport in water is 10 000 times slower than in air (Whalen and Reeburgh, 1992; Castro *et al*., 1995). In contrast, when moisture content is very low (< 12% v/v), CH_4 consumption is also inhibited as observed in desert and landfill cover soils (Whalen *et al*., 1990; Striegl *et al*., 1992). It was suggested that inhibition of atmospheric CH_4 oxidation was caused by increased osmotic stress and desiccation (Conrad, 1996; Jäckel *et al*., 2001). The effects of temperature on CH_4 oxidation are inconsistent, indicating that soil methanotrophs may adapt to different temperatures (Hanson and Hanson, 1996) maybe because they thrive over a large range of temperatures (Table 2). Although temperature changes had little effect on overall CH_4 consumption, temperatures < 10°C and > 40°C seem to decrease significantly methanotrophic activity in forest and landfill cover soils, possibly due to the inhibition of mesophilic methanotroph activity (Castro *et al*., 1995; Semrau *et al*., 2010). Interestingly, methanogens are highly sensitive to variations in temperature and pH (discussed by Le Mer and Roger, 2001). Thus, they may influence more the net CH_4 flux than any other abiotic factors affecting methanogenesis. Similarly to temperature, soil pH does not seem to be a strong controller of CH_4 oxidation since comparable values were measured between pH 3.5 and pH 8 (Born *et al*., 1990). Again, methanotrophs seem to be adapted to a range of soil pH due to the existence of acido-, neutro- and

alkaliphiles (Table 2). However, soil alkalinity and acidity were found to decrease net CH_4 fluxes in grassland and forest soils (Hütsch *et al*., 1994; Amaral *et al*., 1998; Reay *et al*., 2001). More recently, a meta-analysis on conifer and deciduous forest soils revealed that soil pH can influence the community structure of methanotrophs Kolb (2009). The author observed that gammaproteobacteria-related methanotrophs and members of the upland soil cluster γ (USCγ) were predominantly found in neutral soils, while alphaproteobacteria-related methanotrophs and members of the USCα thrived in acidic soils, suggesting clear niche adaptations. Also, the recent discovery of *Methylacidiphilum* proved that CH_4 consumption at very acidic pH (< 1) is possible (Op den Camp *et al*., 2009). A number of articles have provided in-depth reviews of soil methanogenesis and its role in CH_4 flux and reported a strong impact of temperature and soil pH on both methanogens and methanogenesis (Chin *et al*., 1999; Le Mer and Roger, 2001; Conrad *et al*., 2006; Conrad, 2009).

Soil characteristics such as porosity, WFPS and bulk density are closely linked to soil water content and therefore influence O_2 availability and CH_4 diffusion in soils (Ball *et al*., 1997; Le Mer and Roger, 2001; Smith *et al*., 2003). Soil texture and mineralogy are believed to be strong regulators of CH_4 flux (Castro *et al*., 1995; Le Mer and Roger, 2001). Soils containing some types of clay protect organic matter from mineralization (Oades, 1988) and soils with high clay content can retain CH_4 by preventing diffusion (Sass *et al*., 1994). In summary, soil moisture, WFPS and porosity are recognized as important drivers of CH_4 flux rates. Temperature has a positive impact on methanogenesis but is not a major driver of methanotrophy. Elevated temperature and levels of CO_2 may have direct and/or indirect effect on CH_4 flux and global warming, as discussed previously (Singh *et al*., 2010).

Study of the ecology and niche adaptation of methanotrophs/methanogens is still in its infancy and further research is necessary to unravel these important aspects of the ecology of functional microbial communities. A recent report confirmed that methanogens are ubiquitous in aerobic soils and can actively produce CH_4 as soon as conditions change to anoxic (Angel *et al*., 2012). The authors concluded that *Methanosarcina* and *Methanocella* appear to be universal members of the biomes in aerated soils. Niche partitioning among methanogenic *Archaea* has also been observed. Kemnitz and colleagues (2004) found that soils which are never or rarely flooded were dominated by *Crenarchaeota*, *Methanomicrobiaceae*, *Methanosarcinaceae*. Uncultivable Rice cluster IV and VI were present in frequently and permanently flooded soils, while *Methanosaetaceae* was detected only in soils which were frequently or permanently flooded and where the acetate concentration was < 30 μm, which indicated this taxon is particularly adapted for low acetate concentration under severe anoxic conditions (Kemnitz *et al*., 2004). This would suggest strong niche adaptation of methanogens under field conditions. However, in-depth studies are required to define ecological niches for different methanogens explicitly considering abiotic environmental drivers, land-use history and management practices, and climatic conditions.

Ho and colleagues (2013) tried to conceptualize how methanotrophs could adopt differing life strategies based on their functional and ecological characteristics. Using a three-dimensional (Competitor–Stress tolerator–Ruderal) functional classification framework, the authors suggested that gammaproteobacterial methanotrophs are composed of competitor and ruderal species because they respond rapidly to disturbances and are usually active in many environments. Alternatively, alphaproteobacterial methanotrophs are stress tolerant species due to their persistence, stability and ability to use versatile substrates, in response to fluctuating environmental conditions (Ho *et al*., 2013). Further studies to demonstrate if existing ecological theories, usually applied to macroorganisms such as plants (Tilman *et al*., 2006), can explain microbial regulation of CH_4 fluxes are also needed. No such study has been reported for methanogenesis. Limited evidence is available in the literature for methanotrophy and is contradictory. For example, Levine and colleagues (2011) demonstrated that the complimentary theory explains CH_4 fluxes, i.e. that an increase in biodiversity (e.g. methanotroph diversity) increases function (e.g. CH_4 oxidation) but other reports contradict this finding. It was suggested that a few dominant species regulate methanotrophy in soils (Singh *et al*., 2007; Kolb, 2009; Nazaries *et al*., 2011), which may suggest that the selection theory (only a selected number of species performs the main function) better explains methanotrophy (Nazaries *et al*., 2013). It is possible that both theories can explain CH_4 flux but in different environmental conditions. For example, in disturbed sites, the complementary theory may be more applicable because both community and environmental conditions are undergoing change and, as a result, higher number of species will be allowed to occupy a large number of niches, which in turn will ensure greater activity [gammaproteobacterial methanotrophs are competitors–ruderals as in the Competitor–Stress tolerator–Ruderal concept (Ho *et al*., 2013)]. On the other hand, in established ecosystem, niches are well defined and one or few species will occupy those niches and therefore, will control the physiological activities and CH_4 flux. However, distinguishing which theory (complementary versus selection) explains microbial regulation of CH_4 flux at the global scale is key information for both incorporation of microbial data in predictive models and future conservation policy on soil biodiversity.

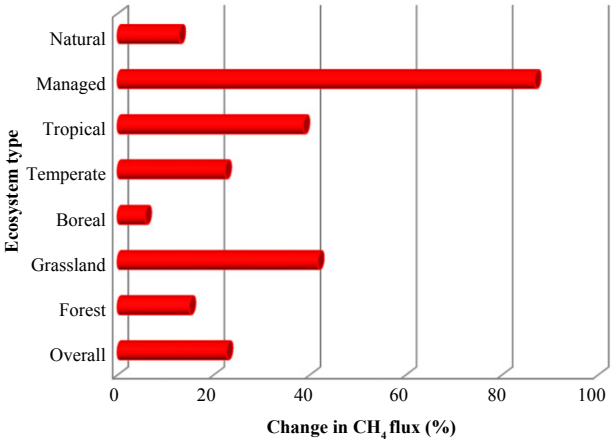

Fig. 3. Effect of N-fertilizer addition on CH_4 flux in different types of ecosystems. The raw data used to produce this figure were provided by Aronson and Helliker (2010). Data indicate that nitrogen-based fertilizers increase CH_4 emissions in all ecosystems.

Effect of change in land management and land use

Influence of land management. The use of inorganic/organic fertilizers is an important practice for land management. Conflicting reports on the precise effect of nitrogenous fertilizers (ammonia- or nitrate-based) on CH_4 flux in soils exist, showing either no effect, inhibition or stimulation (Bodelier and Laanbroek, 2004; Mohanty *et al*., 2006). However, several studies have shown that inorganic fertilizers inhibit methanotrophs while stimulate methanogens in many environments such as rice paddies, grassland and forests (Conrad and Rothfuss, 1991; Hütsch *et al*., 1994; Hanson and Hanson, 1996). This is attributed to possible competitive inhibition of MMO by ammonium (NH_4^+), or through the toxicity of nitrate (NO_3^-) (Bodelier, 2011). Long-term application of NH_4^+-N fertilizer decreased soil CH_4 sink strength, whereas NO_3^--N did not (Willison *et al*., 1995). Conversely, organic fertilizers (compost but not manure) had no effect on methanotrophy in comparison with inorganic fertilizers (Seghers *et al*., 2005). Thus, the form of fertilizer (organic versus inorganic) as well as the form of N (NH_4^+-N versus NO_3^--N) are important in determining rates of CH_4 sink potential.

It is well documented that nitrate addition suppresses methanogenesis in soils (Klüber and Conrad, 1998) by either competition or toxic effects (Conrad, 2007). Competitive suppression of methanogenesis by nitrate is believed to be affected by the nitrate-induced release of sulfate and ferric ions and thus allowing successful competition from sulfate and iron reducers for available H_2 – an important substrate for CH_4 production (Klüber and Conrad, 1998). An alternative mechanism of nitrate-induced inhibition of methanogenesis proposes that intermediate products of denitrification (nitrite, nitric oxide and N_2O) can be toxic to all microbes including methanogens (Roy and Conrad, 1999). However, suppression of CH_4 emissions due to the application of nitrate-based fertilizers has not been reported under field conditions. It is argued that this is because nitrate is either rapidly assimilated by plants or denitrified and released in the atmosphere (Conrad, 2007). However, most of the literature suggests that ammonium-based fertilizers (e.g. urea) may decrease CH_4 emission from soils; however, the mechanism of this inhibition is not known but may occur due to its direct impact on methanogenesis or methanotrophy. Indirectly, fertilizers can increase plant productivity, which in turn increases the supply of organic substrates for methanogenesis (Conrad, 2007; Conrad *et al*., 2008).

In a meta-analysis, Liu and Greaver (2009) estimated that N-fertilizers (urea, NH_4^+ and NO_3^-) application could cause a 97% increase in CH_4 emission and 38% decrease in CH_4 uptake by soils across a range of non-forest and forest ecosystems. In another meta-analysis of 33 studies, Aronson and Helliker (2010) reported that lower N applications could stimulate CH_4 uptake and thus reduce CH_4 emissions. However, the addition of > 100 kg N ha^{-1} $year^{-1}$ (urea, NH_4^+ or NO_3^-) resulted in significant inhibition of CH_4 uptake and the consequent increase in CH_4 emissions in all ecosystems (Fig. 3). This inhibition was dependent on type of fertilizer, rate, biome and management practices (Aronson and Helliker, 2010). Microbial investigations suggest that gammaproteobacterial methanotrophs, which are more active in wetlands, are stimulated by the addition of N-fertilizers (Qiu *et al*., 2008; Shrestha *et al*., 2010). On the other hand, the activity of alphaproteobacterial methanotrophs, which are dominant in upland soils, could be inhibited by N additions (Mohanty *et al*., 2006). Recent work based on the meta-analysis of 33 published studies suggested that competitive inhibition of methanotrophs was not a dominant mechanism controlling CH_4 emissions from N-fertilized fields. It was concluded that CH_4 emissions were more strongly influenced by fertilizer-induced crop yields and simultaneous increase in the release of carbon substrates for methanogenesis (Banger *et al*., 2012). Overall, the effects of the application of nitrogen-based compounds on methanotrophy and methanogenesis are controversial and further understanding is required, in particular at the molecular level.

The effects of biocides (insecticides, pesticides and herbicides) on CH_4 flux were also investigated. Some lowered CH_4 oxidation rates (Mosier *et al*., 1991; Topp, 1993; Boeckx *et al*., 1998; Priemé and Ekelund, 2001) whereas others did not have an effect (Hütsch, 1996; Seghers *et al*., 2003b; 2005). Few studies evaluated the impact of fertilizer and herbicides on abundance and community structure of methanogens and methanotrophs. Seghers and colleagues (2003a,b; 2005) concluded that

application of organic fertilizer improved CH_4 oxidation rates and the abundance of methanotrophs, in particular alphaproteobacterial methanotrophs, whereas the combined application of fertilizers and herbicides had no effect. The herbicide Butachlor was reported to inhibit both methanogenesis and methanotrophy, indicating that some herbicides may have an impact on the soil CH_4 cycle (Mohanty *et al*., 2004). Other agricultural practices such as tillage and ploughing can reduce CH_4 sink, although tillage seems to be less damaging while direct seeding improve CH_4 oxidation (Dalal *et al*., 2008).

Influence of land use. Changes in land use, such as afforestation and deforestation, can influence the CH_4 source and sink strength. From several studies, the following trend can be observed (in decreasing order of sink strength): woodland > non-cultivated upland > grassland > cultivated soils (Hütsch *et al*., 1994; Willison *et al*., 1995; Dobbie and Smith, 1996; Le Mer and Roger, 2001). More specifically, an effect of land-use change on CH_4 sink rates was associated with tree species (Reay *et al*., 2001; Menyailo and Hungate, 2003; Borken and Beese, 2006; Saggar *et al*., 2007; Menyailo *et al*., 2010). Different tree species have varied effects on CH_4 sinks but a common trend was that soils under hardwood species (aspen, beech, birch, oak) consumed more CH_4 than soils under coniferous species (larch, pine, spruce). Interestingly, other studies observed that soils under alder did not oxidize significant amounts of CH_4 (Reay *et al*., 2001; 2005). This was correlated with high nitrification rates and it was suggested that the high concentrations of nitrate measured were the result of N_2 fixation by the symbiotic *Frankia* sp. in the root nodules and subsequent conversion of ammonia to nitrate by ammonia-oxidizing bacteria.

Arable soils are mainly considered as a net source of CH_4 due to the impact of farming practices and use of fertilizers on soil abiotic and biotic characteristics (IPCC, 2007). Deforestation and conversion of forest and grassland to arable soils are known to increase CH_4 emission from soils (Zerva and Mencuccini, 2005; Tate *et al*., 2006; Dörr *et al*., 2010). Recently, a study suggested that land-use change from upland (soybean) cultivation to long-term rice cropping also significantly increased CH_4 emission and this change in emission was linked to a lower (iron) electron availability in rice fields (Eusufzai *et al*., 2010). Compared with arable soils, set-aside soils shows little or no difference in CH_4 oxidation rates, indicating that recovery from agricultural use is not immediate (Dobbie and Smith, 1996; Hütsch, 1998). A study reported that, in Denmark and Scotland, over 100 years were necessary to reach pre-cultivation levels of CH_4 oxidation rates after land-use change from agriculture to woodland (Priemé *et al*., 1997). Modelling studies supported this estimate for all Northern European soils (Smith *et al*., 2000). However, mechanisms that regulate recovery are still debated. Afforested soils showed increased uptake of atmospheric CH_4, due partly to a change in soil abiotic properties (Hiltbrunner *et al*., 2012), and party to a shift in the community structure from type I to type II methanotrophs when shifting land use from pasture to forests (Singh *et al*., 2007; 2009). In particular, there is evidence which suggest that increased CH_4 sinks following afforestation/reforestation were associated with an increased dominance of particular methanotrophs, members of USCα clade, distantly related to type II methanotrophs (Nazaries, 2011; Nazaries *et al*., 2011). This was observed in sites spread across New Zealand and Scotland (Singh *et al*., 2007; Nazaries *et al*., 2011; 2013). In contrast, forest clear-felling and deforestation changed the soil from being a sink for CH_4 into a net source (Keller *et al*., 1990; Keller and Reiners, 1994; Zerva and Mencuccini, 2005). This change was linked to a shift from type II methanotrophs of the *Beijerinckiaceae* towards type I methanotrophs of the *Methylococcaceae* occurring after conversion of forest to farmland (Dörr *et al*., 2010). However, none of these studies included analyses of methanogens, the population of which may have also shifted and could influence the rate of CH_4 flux independently and in association with methanotrophs. A relationship between methanogen activity and methanogenesis was reported in peat soils (Freitag and Prosser, 2009) and was further correlated with the ratio of methanogen and methanotroph activities with CH_4 flux rate (Freitag *et al*., 2010).

It can be assumed that changes in methanotrophic/methanogenic activity and atmospheric CH_4 sinks are also driven by environmental and ecological factors. Lower CH_4 flux has been linked to higher CH_4 uptake and lower production rates, which were mostly correlated with lower water content and lack of chemical fertilization in soils and sediments (Hiltbrunner *et al*., 2012). In addition, competition between alpha- and gammaproteobacterial methanotrophs occurs through copper, CH_4, O_2 and nitrogen availability in soils. The findings above will have a significant impact on future global methane emissions as arable land area is set to increase and management practices will change in the future to meet human needs for food (Nelson *et al*., 2010).

Climate change and its impact on the methane cycle and activities of methanotrophs and methanogens

The vast majority of the literature suggests that climate change will increase global CH_4 fluxes. Increasing concentrations of CO_2 and elevated temperature both impact a number of soil biotic and abiotic characteristics, including the activities of methanogens and methanotrophs, and water content in soil pores (van Groenigen *et al*., 2011).

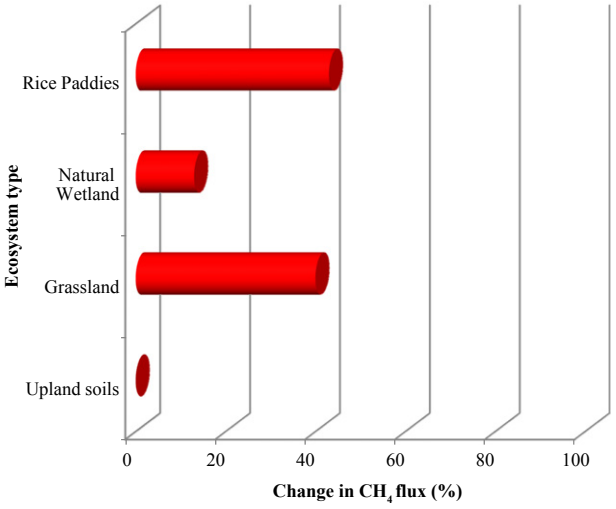

Fig. 4. Effect of elevated CO_2 concentrations on CH_4 flux in different types of ecosystems. Data were obtained from a number of published literatures. A significant portion was obtained from van Groenigen and colleagues (2011) and Ineson and colleagues (1998). Available data suggest that elevated CO_2 increases CH_4 emissions in all ecosystems except in upland soils.

These changes in soil properties will have both direct and indirect impacts on CH_4 production and consumption. In a recent meta-analysis, van Groenigen and colleagues (2011) estimated a significant increase in CH_4 emissions under elevated CO_2 from both natural and managed wetlands including rice paddies, which together are estimated to contribute to 53% of total global CH_4 emissions (Fig. 4). No change in upland CH_4 consumption was predicted (Fig. 4). van Groenigen and colleagues (2011) also estimated that increased production of CH_4 and N_2O under elevated CO_2 conditions will negate up to 17% of the climate change mitigation potential previously predicted for terrestrial carbon sink under future atmospheric CO_2 concentration. Previous studies suggested up to 30% reduction in CH_4 consumption by temperate forest soils in response to increasing atmospheric CO_2 concentration, which is equivalent to a 10% reduction in the sink strength of temperate forest soils (Phillips *et al.*, 2001). Similarly, more than 50% decrease in CH_4 consumption in grassland under elevated CO_2 was reported (Ineson *et al.*, 1998) (Fig. 4).

There are several mechanisms to explain increased CH_4 emission from soil under elevated CO_2 (Singh *et al.*, 2010). For example, plant-mediated increases in soil moisture due to changed transpiration rates can have an impact on O_2 and CH_4 availability, which in turn will favour methanogenesis over methanotrophy. Additionally, elevated CO_2 (eCO_2) increases primary productivity and rhizodeposition, which produce the main substrates for methanogenesis and, in combination with increased anaerobic conditions and elevated temperature (eT), will promote methanogenesis at the expense of methanotrophy. However, contrasting findings on the role of soil moisture in relation to CH_4 flux under eCO_2 have been reported (Singh *et al.*, 2010). It is also possible that change in CH_4 flux under eCO_2 is mediated through an indirect impact on microbial physiology and activity and this has been highlighted in recent reports on feedback responses to climate change (Singh *et al.*, 2010). The effect of warming on CH_4 cycle is less known, both at the process level and at the global scale, although methanotrophy and methanogenesis are mutually expected to increase with rising temperature as a physiological response. However, it has been estimated that up to 78% increase in global CH_4 emission could occur if temperatures increased by 3.4°C (Shindell *et al.*, 2004). Increased temperature was also observed to cause a decline in methanotroph abundance in soil (Mohanty *et al.*, 2007). However, the global effect of elevated temperature on methanotrophy is difficult to predict because different ecosystems will respond differently to climate change (Singh *et al.*, 2010). Soil moisture is arguably one of the most important factors in CH_4 flux (Le Mer and Roger, 2001) as it stimulates methanogenesis and inhibits methanotrophy. This is because elevated temperature increases evaporation and alters rainfall patterns, which results in changed soil moisture content. As a consequence, areas which will become wetter under future climate conditions will have higher moisture, creating anaerobic conditions which increases CH_4 production, and at the same time reduces CH_4 consumption by reducing O_2 availability for methanotrophs. Therefore, future modifications in precipitations (rainfall intensity, pattern, frequency) linked to climate change will have a serious impact on the global CH_4 budget.

The real limitations in estimating feedback responses to climate change have been that most studies have investigated impact of only one climate treatment (eCO_2 or eT). Due to increasing evidence, from both modelling and experimental research, of the interacting effects of climate treatments, it is pertinent that future field-based experimentation should include individual and interactive impacts of at least three major climate variables (CO_2, temperature and precipitations). Additionally, the IPCC (2007) predicted a significant increase in the intensity and frequency of extreme weather events (e.g. flash flooding, extreme drought, prolonged heat waves). Such scenarios necessitate setting up next-generation climate change experiments, which should account not only for climate treatments but also for interacting effects with extreme weather events (Fig. 5). Such design is extremely important for CH_4 cycle because both process and functional microflora are mostly impacted by soil temperature and water levels. Future field studies need to include detailed work on different pathways (both process and microflora)

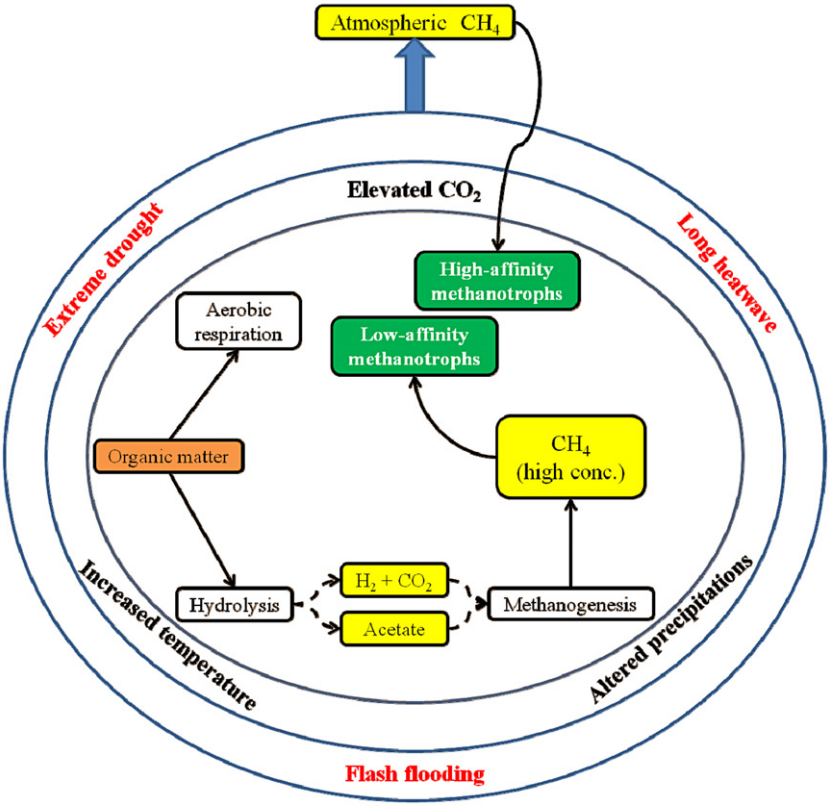

Fig. 5. Conceptual framework for future work for the study of the CH_4 cycle under current and future climate conditions. The response of different pathways for methanogenesis and methanotrophy to global change (land use, management practices) needs to be identified, followed by accurate quantification of change in annual methane flux at the local, regional and global levels. New knowledge on feedback response, i.e. how all these processes will respond to future climatic conditions, is key for accurate prediction of global methane budget. Such experiments should explicitly include (i) individual and interactive impact of climate factors (elevated CO_2, increased temperature and altered precipitations) and (ii) interactions with predicted frequency and intensity of extreme events (flash flooding, extreme drought and prolonged heat waves).

of methanogenesis and methanotrophy and how they will be impacted under individual and interactive climate variables and extreme events (Fig. 5). Quantification of these responses will be key data for the accurate prediction of CH_4 flux under future climatic conditions and for improved national CH_4 inventory.

Modelling

Several predictive models are used to model CH_4 flux and there are significant concerns regarding the predictive values of these models, due to the type and nature of the data used for the development and validation of these models. For example, Glatzel and Bareth (2006) used a bottom-up approach to estimate regional CH_4 emissions, accounting for the interaction between land use and soil type. However, because of the global over-estimation of the results, the authors acknowledged the need to link ecosystem approaches to process-based models, in which specific soil processes such as C and N cycles and their impact on net CH_4 fluxes are considered. The process-based model DNDC (DeNitrification and DeComposition) requires detailed information on site climate (daily precipitation and temperature), soil properties (texture, porosity, clay content, moisture) and land management (crop, fertilization) (Li *et al.*, 1992a; 1992b; Li, 2000). This model was applied to estimate global N_2O emissions from arable sites (Li *et al.*, 1996; Butterbach-Bahl *et al.*, 2001). Recently, the DNDC model was adapted for New Zealand ('NZ-DNDC') for modelling N_2O emissions from grazed pastures (Saggar *et al.*, 2004) and was also successfully applied to model CH_4 consumption (Saggar *et al.*, 2007). However, a consensus exists for these estimations by predictive models that need to incorporate biological processes and microbial communities (Davidson and Janssens, 2006; Singh *et al.*, 2010). No current models account for the role of microorganisms in CH_4 flux. Currently, temperature, precipitation and soil moisture are used to predict CH_4 emissions. Microbial data are not considered due to the complexity of the soil microbial communities which are regarded as a black box in the majority of global change models (Bodelier, 2011). This is because microbes are incredibly diverse, as well as temporally and spatially dynamic. Many microbes are not cultivable under current laboratory regimes and therefore their physiological capabilities are unknown. The above issues make the parameterization of microbial data challenging. However, including microbial data in models for improved prediction of CH_4 flux from terrestrial ecosystems are necessary for the following reasons. (i) Soil methane flux seems to be strongly linked to functional microbial communities. (ii) Current models assume that changes in CH_4 flux are related to changes in soil abiotic properties without considering changes in the

microbial communities. However, since most studies suggest that global change has a direct impact on soil microbial community composition (Singh *et al*., 2010; Kim *et al*., 2012; Nie *et al*., 2013), current models cannot predict changes in flux rates associated with changes in the physiological capabilities of the ecosystem also associated with microbial communities. (iii) It is argued that if historical temperature and precipitations play a role in the ability of microbial adaptations then incorporating microbial data into global change model will be essential to improve their predictions (Docherty and Gutknecht, 2012; Wallenstein and Hall, 2012). (iv) Ecological functions that are carried out by specific and specialized group of microbes, as in the case of CH_4 production and consumption, will be more sensitive to a change in community composition (Schimel and Gulledge, 2004; Singh *et al*., 2010). This makes data (gene expression/abundance, PLFA profile, etc.) from methanogens and methanotrophs ideal for parameterization and their incorporation into predictive models.

The above discussion provides compelling reasons to include microbial data; however, to successfully parameterize and include microbial data in models for a better predictive value, several coordinated steps will be required. First of all, modellers will need long-term data on the global change impact on microbial community, diversity and functions. This is key for the successful inclusion of microbial data into predictive models, because on the short term, soil moisture and temperature can influence the process rate by influencing the metabolic rates of existing microbes. However, on the long term, microbes can adapt to changing climate and reduce the process rates to near ambient conditions (Docherty and Gutknecht, 2012). There is already evidence on acclimation of microbial respiration to increasing temperature (Bradford *et al*., 2008) but we do not know if the same is true for CH_4 flux. Second, microbial data need to be detailed and should include the time needed to determine environmental factors that influence community composition or metabolic rates and the time needed for such change to occur. Finally, data from multifactorial treatments in field and glasshouse experimental procedures need to be detailed. Not only do modellers need microbial process data from integrated global change experiments including land-use change, N deposition and climate change in combination with extreme weathers (Fig. 5) but they also require data at the genetic and expression level. Most of the genes involved in CH_4 cycle are well characterized. Emerging -omic technologies (metagenomics, transcriptomics and proteomics) provide unprecedented opportunities to (semi)-quantitatively characterize shifts in total community, active communities and physiological capabilities of an ecosystem (Box 1). These technologies also have the ability to distinguish treatment effects from spatial and temporal dynamics within microbial communities, which is key for upscaling these data to the ecosystem level.

Current limitations and future perspectives

i. New technologies are still limited by DNA extraction protocols, PCR, sequencing and probe biases, as well as a lack of bioinformatics support for next-generation sequencing and metaproteomics. However, this field has seen a remarkable progress (revolution) in the last 5 years and as the bioinformatic tools improve, the next challenge will be to generate quantitative data for microbes involved in the CH_4 cycle and to parameterize these data for meaningful use in climate/ecosystem models.
ii. Many methanogens and methanotrophs are uncultivable and consequently little is known about their metabolic capabilities. This is a key requirement for successful inclusion of microbial data into the predictive models. Techniques such as DNA- and RNA-stable isotope probing (SIP) can help identifying the physiological capabilities of individual species. There are potential limitations with DNA- and RNA-SIP techniques as relatively high concentrations of substrate need to be used to achieve sufficient labelling of DNA (discussed in Neufeld *et al*., 2007a; 2008). SIP combined with PLFA (PLFA-SIP) can detect active microbes at environmentally relevant concentration. However, this approach lacks phylogenetic resolution to accurately identify microorganisms at the species level. Technological advances in SIP and complementary technology are now being made where environmentally relevant concentrations of substrate can be used for metagenomic and metaproteomic work (for a comprehensive review see Chen and Murrell, 2011; Murrell and Whiteley, 2011).
iii. Conrad (2007) highlighted that it is now possible to describe macroscopic events (such as temporal change in CH_4 flux after fertilization or flooding) by analysing methanogens and methanotrophs. This provides direct evidence of microscopic (microbial) control of macroscopic events. Further evidence will provide strong basis to incorporate both abiotic and biotic data into process-based models and make global predictions (Conrad, 2007). But further evidence will be needed to persuade modellers to utilize parameterized microbial data into simulations (Box 1).
iv. Identifying niches for populations of methanogens and methanotrophs is needed. There is evidence for niche adaptation in methanogens (Yavitt *et al*., 2012) and methanotrophs (Kumaresan *et al*., 2011; Nazaries *et al*., 2011). Recently, Reim and colleagues (2012) detected vertical niche differentiation in gammaproteobacterial methanotrophs within the first

three millimetres of water-saturated soils. This is quite remarkable and signifies the need for detailed niche identification since community change can be observed on such small scale. If we can identify niche separation for specific microbial groups with defined physiological capabilities, this may substantially aid with the incorporation of microbial data into predictive models. This can be partly achieved by accounting for the functional traits and ecological characteristics of methanotrophs (Ho *et al*., 2013), as well as information on methanotroph community structure and diversity in different environments. For example, there is growing evidence that high-affinity methanotrophs of the USCα dominate in forest soils across the globe and are responsible for most of atmospheric CH_4 consumption (Nazaries, 2011). Also, further information on how the physiology of members of the USCα is impacted under future climate conditions and by extreme weather along with the ecology of other important methanogens and methanotrophs will provide the necessary boost in this area of science. This can be achieved by the systematic survey of methanogens and methanotrophs across ecosystems at the regional, national and global scale. Many developed countries now have established soil survey programmes, such as in Scotland, the Netherlands, France, the UK, and inclusion of methanotroph and methanogen analyses will help with the provision of this critical data and the identification of niches for specific groups of microbes.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

1. Methane-production pathways.
2. Differences between aerobic methanotrophs.
3. sMMO versus pMMO.

Fig. S1. Major pathways of CH_4 production in methanogens. The hydrogenotrophic (CO_2 reduction) (A) and aceticlastic (acetate conversion) (B) methanogenesis pathways represent the two major pathways for CH_4 production by methanogens, whereas methylotrophic methanogenesis (utilization of methyl groups) only has a minor contribution (not shown here) (Ferry, 2010). Abbreviations: Ac-CoA, acetyl-coenzyme A; AcS/CODH, acetyl-CoA synthase and carbon monoxide dehydrogenase; AK, acetate kinase; CoB, coenzyme B; CoM, coenzyme M; ECH, energy-converting hydrogenase; F_{420}, coenzyme F_{420}; Fd, ferredoxin; FDH, formate dehydrogenase; H_4MPT, tetrahydromethanopterin; H_4SPT, tetrahydrosarcinapterin; HDR, heterodisulfide reductase; MCR, methyl-coenzyme M reductase; MFR, methanofuran; MTR, methyltransferase. Adapted from Liu and Whitman (2008).

Fig. S2. Oxidation of CH_4 and simplified pathways of carbon assimilation in methanotrophs: (A) RuMP pathway of gammaproteobacterial methanotrophs; (B) serine pathway of alphaproteobacterial methanotrophs; (C) CO_2 fixation via the Calvin–Benson–Bassham cycle. Multiple arrows represent consecutives enzymatic reactions which are not detailed here. Abbreviations: Ac-CoA, acetyl-coenzyme A; CytC, cytochrome C; FalDH, formaldehyde dehydrogenase; FDH, formate dehydrogenase; F-1,6-BP, fructose-1,6-bisphosphate; G-6-P, glucose-6-phosphate; GAP, glyceraldehydes-3-P; GK, glycerate kinase; HPI, hexulose-6-P isomerase; HPS, hexulose-6-P synthase; MDH, methanol dehydrogenase; Pyr, pyruvate; PDH, pyruvate dehydrogenase; R-5-P, ribulose-5-P; R-1,5-BP, ribulose-1,5-bisphosphate; SGAT, serine-glyoxylate aminotransferase; SHTM, serine-hydroxytransmethylase; 2-P-G, 2-phosphoglycerate.

Fig. S3. Physical map of the sMMO operon from *Methylococcus capsulatus* (Bath). The genes encoding for the sMMO enzyme (*mmoXYBZDC*) are highlighted in red. The regulatory genes, *mmoG* and *mmoR* are highlighted in green and purple respectively. The genes encoding for a two-component senor-regulatory system are highlighted in blue. The location and direction of transcription from the σ^{54} promoter is indicated with a black arrow. The gene boundaries are shown to scale and are indicated by the scale bar. Source: Ali (2006).

Fig. S4. Structural gene organization of the pMMO operon in methanotrophs. The *pmoCAB* operon encodes for the α-, β- and γ-subunits of pMMO respectively. The location and direction of transcription from the σ^{70} promoter is indicated with the black arrow. The gene boundaries are shown to scale and are indicated by the scale bar. Source: Ali (2006).